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| (54) Title: IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND PRODUCTION OF VIRUS-LIKE PARTICLES | | |
| (57) Abstract <p>The present invention relates to the efficient expression of HIV polypeptides in a variety of cell types, including, but not limited to, mammalian, insect, and plant cells. Synthetic expression cassettes encoding the HIV Gag-containing polypeptides are described, as are uses of the expression cassettes in applications including DNA immunization, generation of packaging cell lines, and production of Env-, tat- or Gag-containing proteins. The invention provides methods of producing Virus-Like Particles (VLPs), as well as, uses of the VLPs including, but not limited to, vehicles for the presentation of antigens and stimulation of immune response in subjects to whom the VLPs are administered.</p> | | |

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IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND
PRODUCTION OF VIRUS-LIKE PARTICLES

5 **TECHNICAL FIELD**

Synthetic expression cassettes encoding the HIV polypeptides (e.g., Gag-, pol-, prot-, reverse transcriptase, Env- or tat-containing polypeptides) are described, as are uses of the expression cassettes. The present invention relates to the efficient expression of HIV polypeptides in a variety of cell types. Further, the invention provides methods of producing Virus-Like Particles (VLPs), as well as, uses of the VLPs and high level expression of oligomeric envelope proteins.

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BACKGROUND OF THE INVENTION

Acquired immune deficiency syndrome (AIDS) is recognized as one of the greatest health threats facing modern medicine. There is, as yet, no cure for this disease.

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In 1983-1984, three groups independently identified the suspected etiological agent of AIDS. See, e.g., Barre-Sinoussi et al. (1983) Science 220:868-871; Montagnier et al., in Human T-Cell Leukemia Viruses (Gallo, Essex & Gross, eds., 1984); Vilmer et al. (1984) The Lancet 1:753; Popovic et al. (1984) Science 224:497-500; Levy et al. (1984) Science 225:840-842. These isolates were variously called lymphadenopathy-associated virus (LAV), human T-cell lymphotropic virus

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type III (HTLV-III), or AIDS-associated retrovirus (ARV). All of these isolates are strains of the same virus, and were later collectively named Human Immunodeficiency Virus (HIV). With the isolation of a related

5 AIDS-causing virus, the strains originally called HIV are now termed HIV-1 and the related virus is called HIV-2. See, e.g., Guyader et al. (1987) *Nature* 326:662-669; Brun-Vezinet et al. (1986) *Science* 233:343-346; Clavel et al. (1986) *Nature* 324:691-695.

10 A great deal of information has been gathered about the HIV virus, however, to date an effective vaccine has not been identified. Several targets for vaccine development have been examined including the *env*, *Gag*, *pol* and *tat* gene products encoded by HIV.

15 Haas, et al., (*Current Biology* 6(3):315-324, 1996) suggested that selective codon usage by HIV-1 appeared to account for a substantial fraction of the inefficiency of viral protein synthesis. Andre, et al., (*J. Virol.* 72(2):1497-1503, 1998) described an increased immune
20 response elicited by DNA vaccination employing a synthetic gp120 sequence with optimized codon usage. Schneider, et al., (*J Virol.* 71(7):4892-4903, 1997) discuss inactivation of inhibitory (or instability) elements (INS) located within the coding sequences of the
25 *Gag* and *Gag*-protease coding sequences.

The *Gag* proteins of HIV-1 are necessary for the assembly of virus-like particles. HIV-1 *Gag* proteins are involved in many stages of the life cycle of the virus including, assembly, virion maturation after particle
30 release, and early post-entry steps in virus replication. The roles of HIV-1 *Gag* proteins are numerous and complex (Freed, E.O., *Virology* 251:1-15, 1998).

Wolf, et al., (PCT International Application, WO 96/30523, published 3 October 1996; European Patent Application, Publication No. 0 449 116 A1, published 2 October 1991) have described the use of altered pr55 Gag of HIV-1 to act as a non-infectious retroviral-like particulate carrier, in particular, for the presentation of immunologically important epitopes. Wang, et al., (Virology 200:524-534, 1994) describe a system to study assembly of HIV Gag- β -galactosidase fusion proteins into virions. They describe the construction of sequences encoding HIV Gag- β -galactosidase fusion proteins, the expression of such sequences in the presence of HIV Gag proteins, and assembly of these proteins into virus particles.

Recently, Shiver, et al., (PCT International Application, WO 98/34640, published 13 August 1998) described altering HIV-1 (CAM1) Gag coding sequences to produce synthetic DNA molecules encoding HIV Gag and modifications of HIV Gag. The codons of the synthetic molecules were codons preferred by a projected host cell.

The envelope protein of HIV-1 is a glycoprotein of about 160 kD (gp160). During virus infection of the host cell, gp160 is cleaved by host cell proteases to form gp120 and the integral membrane protein, gp41. The gp41 portion is anchored in (and spans) the membrane bilayer of virion, while the gp120 segment protrudes into the surrounding environment. As there is no covalent attachment between gp120 and gp41, free gp120 is released from the surface of virions and infected cells.

Haas, et al., (Current Biology 6(3):315-324, 1996) suggested that selective codon usage by HIV-1 appeared to account for a substantial fraction of the inefficiency of viral protein synthesis. Andre, et al., (J. Virol.

72(2):1497-1503, 1998) described an increased immune response elicited by DNA vaccination employing a synthetic gp120 sequence with optimized codon usage.

5 **SUMMARY OF THE INVENTION**

The present invention relates to improved expression of HIV *Env*-, *tat*-, *pol*-, *prot*-, *reverse transcriptase*, or *Gag*-containing polypeptides and production of virus-like particles.

10 In one embodiment the present invention includes an expression cassette, comprising a polynucleotide encoding an HIV *Gag* polypeptide comprising a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:20. In certain embodiments, the polynucleotide
15 sequence encoding said *Gag* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:9 or SEQ ID NO:4. The expression cassettes may further include a polynucleotide sequence encoding an HIV *protease* polypeptide, for
20 example a nucleotide sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:78, and SEQ ID NO:79. The expression cassettes may further include a polynucleotide sequence encoding an HIV *reverse*
25 *transcriptase* polypeptide, for example a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, and SEQ ID NO:84. The expression cassettes may further include a polynucleotide
30 sequence encoding an HIV *tat* polypeptide, for example a sequence selected from the group consisting of: SEQ ID NO:87, SEQ ID NO:88, and SEQ ID NO:89. The expression cassettes may further include a polynucleotide sequence encoding an HIV *polymerase* polypeptide, for example a

sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:6. The expression cassettes may include a polynucleotide sequence encoding an HIV polymerase polypeptide, wherein (i) the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:4, and (ii) wherein the sequence is modified by deletions of coding regions corresponding to reverse transcriptase and integrase. The expression cassettes described above may preserve T-helper cell and CTL epitopes. The expression cassettes may further include a polynucleotide sequence encoding an HCV core polypeptide, for example a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:7.

In another aspect, the invention includes an expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV Env polypeptide, wherein the polynucleotide sequence encoding said Env polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:71 (Figure 58) or SEQ ID NO:72 (Figure 59). In certain embodiments, the Env expression cassettes include sequences flanking a V1 region but have a deletion in the V1 region itself, for example the sequence presented as SEQ ID NO:65 (Figure 52, gp160.modUS4.delV1). In certain embodiments, the Env expression cassettes include sequences flanking a V2 region but have a deletion in the V2 region itself, for example the sequences shown in SEQ ID NO:60 (Figure 47); SEQ ID NO:66 (Figure 53); SEQ ID NO:34 (Figure 20); SEQ ID NO:37 (Figure 24); SEQ ID NO:40 (Figure 27); SEQ ID NO:43 (Figure 30); SEQ ID NO:46 (Figure 33); SEQ ID NO:76 (Figure 64) and SEQ ID NO:49 (Figure 36). In certain

embodiments, the Env expression cassettes include sequences flanking a V1/V2 region but have a deletion in the V1/V2 region itself, for example, SEQ ID NO:59 (Figure 46); SEQ ID NO:61 (Figure 48); SEQ ID NO:67 (Figure 54); SEQ ID NO:75 (Figure 63); SEQ ID NO:35 (Figure 21); SEQ ID NO:38 (Figure 25); SEQ ID NO:41 (Figure 28); SEQ ID NO:44 (Figure 31); SEQ ID NO:47 (Figure 34) and SEQ ID NO:50 (Figure 37). The Env-encoding expression cassettes may also include a mutated cleavage site that prevents the cleavage of a gp140 polypeptide into a gp120 polypeptide and a gp41 polypeptide, for example, SEQ ID NO:57 (Figure 44); SEQ ID NO:61 (Figure 48); SEQ ID NO:63 (Figure 50); SEQ ID NO:39 (Figure 26); SEQ ID NO:40 (Figure 27); SEQ ID NO:41 (Figure 28); SEQ ID NO:42 (Figure 29); SEQ ID NO:43 (Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45 (Figure 32); SEQ ID NO:46 (Figure 33); and SEQ ID NO:47 (Figure 34). The Env expression cassettes may include a gp160 Env polypeptide or a polypeptide derived from a gp160 Env polypeptide, for example SEQ ID NO:64 (Figure 51); SEQ ID NO:65 (Figure 52); SEQ ID NO:66 (Figure 53); SEQ ID NO:67 (Figure 54); SEQ ID NO:68 (Figure 55); SEQ ID NO:75 (Figure 63); SEQ ID NO:73 (Figure 61); SEQ ID NO:48 (Figure 35); SEQ ID NO:49 (Figure 36); SEQ ID NO:50 (Figure 37); SEQ ID NO:76 (Figure 64); and SEQ ID NO:74 (Figure 62). The Env expression cassettes may include a gp140 Env polypeptide or a polypeptide derived from a gp140 Env polypeptide, for example SEQ ID NO:56 (Figure 43); SEQ ID NO:57 (Figure 44); SEQ ID NO:58 (Figure 45); SEQ ID NO:59 (Figure 46); SEQ ID NO:60 (Figure 47); SEQ ID NO:61 (Figure 48); SEQ ID NO:62 (Figure 49); SEQ ID NO:63 (Figure 50); SEQ ID NO:36 (Figure 23); SEQ ID NO:37 (Figure 24); SEQ ID NO:38 (Figure 25); SEQ ID NO:39

(Figure 26); SEQ ID NO:40 (Figure 27); SEQ ID NO:41 (Figure 28); SEQ ID NO:42 (Figure 29); SEQ ID NO:43 (Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45 (Figure 32); SEQ ID NO:46 (Figure 33); and SEQ ID NO:47 (Figure 34). The Env expression cassettes may also include a gp120 Env polypeptide or a polypeptide derived from a gp120 Env polypeptide, for example SEQ ID NO:54 (Figure 41); and SEQ ID NO:55 (Figure 42); SEQ ID NO:33 (Figure 19); SEQ ID NO:34 (Figure 20); and SEQ ID NO:35 (Figure 21). The Env expression cassettes may include an Env polypeptide lacking the amino acids corresponding to residues 128 to about 194, relative to strains SF162 or US4, for example, SEQ ID NO:55 (Figure 42); SEQ ID NO:62 (Figure 49); SEQ ID NO:63 (Figure 50); and SEQ ID NO:68 (Figure 55).

In another aspect, the invention includes a recombinant expression system for use in a selected host cell, comprising, one or more of the expression cassettes described herein operably linked to control elements compatible with expression in the selected host cell. The expression cassettes may be included on one or on multiple vectors and may use the same or different promoters. Exemplary control elements include a transcription promoter (e.g., CMV, CMV+intron A, SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein), a transcription enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences.

In another aspect, the invention includes a recombinant expression system for use in a selected host cell, comprising, any one of the expression cassettes described herein operably linked to control elements

compatible with expression in the selected host cell. Exemplary control elements include, but are not limited to, a transcription promoter (e.g., CMV, CMV+intron A, SV40, RSV, HIV-LTR, MMLV-LTR, and metallothionein), a
5 transcription enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences.

In yet another aspect, the invention includes a cell
10 comprising one or more of the expression cassettes described herein operably linked to control elements compatible with expression in the cell. The cell can be, for example, a mammalian cell (e.g., BHK, VERO, HT1080, 293, RD, COS-7, or CHO cells), an insect cell (e.g.,
15 *Trichoplusia ni* (Tn5) or Sf9), a bacterial cell, a plant cell, a yeast cell, an antigen presenting cell (e.g., primary, immortalized or tumor-derived lymphoid cells such as macrophages, monocytes, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof).

20 In another aspect, the invention includes methods for producing a polypeptide including HIV *Gag*-, *prot*-, *pol*-, reverse transcriptase, *Env*- or *Tat*-containing polypeptide sequences, said method comprising, incubating the cells comprising one or more the expression cassettes
25 describe herein, under conditions for producing said polypeptide.

In yet another aspect, the invention includes compositions for generating an immunological response, comprising one or more of the expression cassettes
30 described herein. In certain embodiments, the compositions also include an adjuvant.

In a still further aspect, the invention includes methods of generating an immune response in a subject, comprising introducing a composition comprising one or

more of the expression cassettes described herein into the subject under conditions that are compatible with expression of said expression cassette in the subject.

In certain embodiments, the expression cassette is introduced using a gene delivery vector. More than one expression cassette may be introduced using one or more gene delivery vectors.

In yet another aspect, the invention includes a purified polynucleotide comprising a polynucleotide sequence encoding a polypeptide including an HIV Env polypeptide, wherein the polynucleotide sequence encoding said Env polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:71 (Figure 58) or SEQ ID NO:72 (Figure 59). Further exemplary purified polynucleotide sequences were presented above.

The polynucleotides of the present invention can be produced by recombinant techniques, synthetic techniques, or combinations thereof.

In another embodiment, the invention includes a method for producing a polypeptide including HIV Gag polypeptide sequences, where the method comprises incubating any of the above cells containing an expression cassette of interest under conditions for producing the polypeptide.

The invention further includes, a method for producing virus-like particles (VLPs) where the method comprises incubating any of the above-described cells containing an expression cassette of interest under conditions for producing VLPs.

In another aspect the invention includes a method for producing a composition of virus-like particles (VLPs) where, any of the above-described cells containing an expression cassette of interest are incubated under

conditions for producing VLPs, and the VLPs are substantially purified to produce a composition of VLPs.

In a further embodiment of the present invention, packaging cell lines are produced using the expression cassettes of the present invention. For example, a cell line useful for packaging lentivirus vectors comprises suitable host cells that have an expression vector containing an expression cassette of the present invention wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell. In a preferred embodiment, such host cells may be transfected with one or more expression cassettes having a polynucleotide sequence that encodes an HIV polymerase polypeptide or polypeptides derived therefrom, for example, where the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:6. Further, the HIV polymerase polypeptide may be modified by deletions of coding regions corresponding to reverse transcriptase and integrase. Such a polynucleotide sequence may preserve T-helper cell and CTL epitopes, for example when used in a vaccine application. In addition, the polynucleotide sequence may also include other polypeptides. Further, polynucleotide sequences encoding additional polypeptides whose expression are useful for packaging cell line function may also be utilized.

In another aspect, the present invention includes a gene delivery or vaccine vector for use in a subject, where the vector is a suitable gene delivery vector for use in the subject, and the vector comprises one or more of any of the expression cassettes of the present

invention where the polynucleotide sequences of interest are operably linked to control elements compatible with expression in the subject. Such gene delivery vectors can be used in a method of DNA immunization of a subject, 5 for example, by introducing a gene delivery vector into the subject under conditions that are compatible with expression of the expression cassette in the subject. Gene delivery vectors useful in the practice of the present invention include, but are not limited to, 10 nonviral vectors, bacterial plasmid vectors, viral vectors, particulate carriers (where the vector is coated on a polylactide co-glycolide particles, gold or tungsten particle, for example, the coated particle can be delivered to a subject cell using a gene gun), liposome 15 preparations, and viral vectors (e.g., vectors derived from alphaviruses, pox viruses, and vaccinia viruses, as well as, retroviral vectors, including, but not limited to, lentiviral vectors). Alphavirus-derived vectors include, for example, an alphavirus cDNA construct, a 20 recombinant alphavirus particle preparation and a eukaryotic layered vector initiation system. In one embodiment, the subject is a vertebrate, preferably a mammal, and in a further embodiment the subject is a human.

25 The invention further includes a method of generating an immune response in a subject, where cells of a subject are transfected with any of the above-described gene delivery vectors (e.g., alphavirus constructs; alphavirus cDNA constructs; eukaryotic 30 layered vector initiation systems (see, e.g., U.S. Patent Number 5,814,482 for description of suitable eukaryotic layered vector initiation systems); alphavirus particle

preparations; etc.) under conditions that permit the expression of a selected polynucleotide and production of a polypeptide of interest (i.e., encoded by any expression cassette of the present invention), thereby
5 eliciting an immunological response to the polypeptide. Transfection of the cells may be performed *ex vivo* and the transfected cells are reintroduced into the subject. Alternately, or in addition, the cells may be transfected *in vivo* in the subject. The immune response may be
10 humoral and/or cell-mediated (cellular).

Further embodiments of the present invention include purified polynucleotides. In one embodiment, the purified polynucleotide comprises a polynucleotide sequence having at least 90% sequence identity to the
15 sequence presented as SEQ ID NO:20, and complements thereof. In another embodiment, the purified polynucleotide comprises a polynucleotide sequence encoding an HIV Gag polypeptide, wherein the polynucleotide sequence comprises a sequence having at
20 least 90% sequence identity to the sequence presented as SEQ ID NO:20, and complements thereof. In still another embodiment, the purified polynucleotide comprises a polynucleotide sequence encoding an HIV Gag polypeptide, wherein the polynucleotide sequence comprises a sequence
25 having at least 90% sequence identity to the sequence presented as SEQ ID NO:9, and complements thereof. In further embodiments the polynucleotide sequence comprises a sequence having at least 90% sequence identity to one of the following sequences: SEQ ID NO:4, SEQ ID NO:5, SEQ
30 ID NO:6, SEQ ID NO:7, and complements thereof.

The polynucleotides of the present invention can be produced by recombinant techniques, synthetic techniques, or combinations thereof.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

5 **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 shows the locations of the inactivation sites for the native HIV-1SF2 Gag protein coding sequence.

10 Figure 2 shows the locations of the inactivation sites for the native HIV-1SF2 Gag-protease protein coding sequence.

Figures 3A and 3B show electron micrographs of virus-like particles. Figure 3A shows immature p55Gag virus-like particles in COS-7 cells transfected with a synthetic HIV-1_{SF2} gag construct while Figure 3B shows 15 mature (arrows) and immature VLP in cells transfected with a modified HIV-1_{SF2} gagprotease construct (GP2, SEQ ID NO:70). Transfected cells were fixed at 24 h (gag) or 48 h (gagprotease) post-transfection and subsequently 20 analyzed by electron microscopy (magnification at 100,000X). Cells transfected with vector alone (pCMVKm2) served as negative control (data not shown).

Figure 4 presents an image of samples from a series of fractions which were electrophoresed on an 8-16% SDS 25 polyacrylamide gel and the resulting bands visualized by commassie blue staining. The results show that the native p55 Gag virus-like particles (VLPs) banded at a sucrose density of range of 1.15 - 1.19 g/ml with the peak at approximately 1.17 g/ml.

30 Figure 5 presents an image similar to Figure 4 where the analysis was performed using Gag VLPs produced by a synthetic Gag expression cassette.

Figure 6 presents a comparison of the total amount of purified HIV p55 Gag from several preparations obtained from two baculovirus expression cassettes encoding native and modified Gag.

5 Figure 7 presents an alignment of modified coding sequences of the present invention including a synthetic Gag expression cassette (SEQ ID NO:4), a synthetic Gag-protease expression cassette (SEQ ID NO:5), and a
10 synthetic Gag-polymerase expression cassette (SEQ ID NO:6). A common region (Gag-common; SEQ ID NO:9) extends from position 1 to position 1262.

Figure 8 presents an image of wild-type Gag-HCV core expression samples from a series of fractions which were electrophoresed on an 8-16% SDS polyacrylamide gel and
15 the resulting bands visualized by commassie staining.

Figure 9 shows the results of Western blot analysis of the gel shown presented in Figure 8.

Figure 10 presents results similar to those shown in Figure 9. The results in Figure 10 indicate that the
20 main HCV Core-specific reactivity migrates at an approximate molecular weight of 72,000 kD, which is in accordance with the predicted molecular weight of the Gag-HCV core chimeric protein.

Figures 11A to 11D present a comparison of AT
25 content, in percent, of cDNAs corresponding to an unstable human mRNA (human IFN γ mRNA; 11A), wild-type HIV Gag native RNA (11B), a stable human mRNA (human GAPDH mRNA; 11C), and synthetic HIV Gag RNA (11D).

Figure 12 shows the location of the inactivation
30 sites for the native HIV-1SF2 Gag-polymerase sequence.

Figure 13A presents a vector map of pESN2dhfr.

Figure 13B presents a map of the pCMVIII vector.

Figure 14 presents a vector map of pCMV-LINK.

Figure 15 presents a schematic diagram showing the relationships between the following forms of the HIV Env polypeptide: gp160, gp140, gp120, and gp41.

5 Figure 16 depicts the nucleotide sequence of wild-type gp120 from SF162 (SEQ ID NO:30).

Figure 17 depicts the nucleotide sequence of the wild-type gp140 from SF162 (SEQ ID NO:31).

Figure 18 depicts the nucleotide sequence of the wild-type gp160 from SF162 (SEQ ID NO:32).

10 Figure 19 depicts the nucleotide sequence of the construct designated gp120.modSF162 (SEQ ID NO:33).

Figure 20 depicts the nucleotide sequence of the construct designated gp120.modSF162.delV2 (SEQ ID NO:34).

15 Figure 21 depicts the nucleotide sequence of the construct designated gp120.modSF162.delV1/V2 (SEQ ID NO:35).

Figures 22A-H show the percent A-T content over the length of the sequences for IFN γ (Figures 2C and 2G); native gp160 Env US4 and SF162 (Figures 2A and 2E, respectively); GAPDH (Figures 2D and 2H); and the synthetic gp160 Env for US4 and SF162 (Figures 2B and 2F, respectively).

Figure 23 depicts the nucleotide sequence of the construct designated gp140.modSF162 (SEQ ID NO:36).

25 Figure 24 depicts the nucleotide sequence of the construct designated gp140.modSF162.delV2 (SEQ ID NO:37).

Figure 25 depicts the nucleotide sequence of the construct designated gp140.modSF162.delV1/V2 (SEQ ID NO:38).

30 Figure 26 depicts the nucleotide sequence of the construct designated gp140.mut.modSF162 (SEQ ID NO:39).

Figure 27 depicts the nucleotide sequence of the construct designated gp140.mut.modSF162.delV2 (SEQ ID NO:40).

Figure 28 depicts the nucleotide sequence of the construct designated gp140.mut.modSF162.delV1/V2 (SEQ ID NO:41).

5 Figure 29 depicts the nucleotide sequence of the construct designated gp140.mut7.modSF162 (SEQ ID NO:42).

Figure 30 depicts the nucleotide sequence of the construct designated gp140.mut7.modSF162.delV2 (SEQ ID NO:43).

10 Figure 31 depicts the nucleotide sequence of the construct designated gp140.mut7.modSF162.delV1/V2 (SEQ ID NO:44).

Figure 32 depicts the nucleotide sequence of the construct designated gp140.mut8.modSF162 (SEQ ID NO:45).

15 Figure 33 depicts the nucleotide sequence of the construct designated gp140.mut8.modSF162.delV2 (SEQ ID NO:46).

Figure 34 depicts the nucleotide sequence of the construct designated gp140.mut8.modSF162.delV1/V2 (SEQ ID NO:47).

20 Figure 35 depicts the nucleotide sequence of the construct designated gp160.modSF162 (SEQ ID NO:48).

Figure 36 depicts the nucleotide sequence of the construct designated gp160.modSF162.delV2 (SEQ ID NO:49).

25 Figure 37 depicts the nucleotide sequence of the construct designated gp160.modSF162.delV1/V2 (SEQ ID NO:50).

Figure 38 depicts the nucleotide sequence of the wild-type gp120 from US4 (SEQ ID NO:51).

30 Figure 39 depicts the nucleotide sequence of the wild-type gp140 from US4 (SEQ ID NO:52).

Figure 40 depicts the nucleotide sequence of the wild-type gp160 from US4 (SEQ ID NO:53).

Figure 41 depicts the nucleotide sequence of the construct designated gp120.modUS4 (SEQ ID NO:54).

Figure 42 depicts the nucleotide sequence of the construct designated gp120.modUS4.del 128-194 (SEQ ID NO:55).

5 Figure 43 depicts the nucleotide sequence of the construct designated gp140.modUS4 (SEQ ID NO:56).

Figure 44 depicts the nucleotide sequence of the construct designated gp140.mut.modUS4 (SEQ ID NO:57).

Figure 45 depicts the nucleotide sequence of the construct designated gp140.TM.modUS4 (SEQ ID NO:58).

10 Figure 46 depicts the nucleotide sequence of the construct designated gp140.modUS4.delV1/V2 (SEQ ID NO:59).

Figure 47 depicts the nucleotide sequence of the construct designated gp140.modUS4.delV2 (SEQ ID NO:60).

15 Figure 48 depicts the nucleotide sequence of the construct designated gp140.mut.modUS4.delV1/V2 (SEQ ID NO:61).

Figure 49 depicts the nucleotide sequence of the construct designated gp140.modUS4.del 128-194 (SEQ ID NO:62).

20 Figure 50 depicts the nucleotide sequence of the construct designated gp140.mut.modUS4.del 128-194 (SEQ ID NO:63).

Figure 51 depicts the nucleotide sequence of the construct designated gp160.modUS4 (SEQ ID NO:64).

Figure 52 depicts the nucleotide sequence of the construct designated gp160.modUS4.delV1 (SEQ ID NO:65).

Figure 53 depicts the nucleotide sequence of the construct designated gp160.modUS4.delV2 (SEQ ID NO:66).

30 Figure 54 depicts the nucleotide sequence of the construct designated gp160.modUS4.delV1/V2 (SEQ ID NO:67).

Figure 55 depicts the nucleotide sequence of the construct designated gp160.modUS4.del 128-194 (SEQ ID NO:68).

5 Figure 56 depicts the nucleotide sequence of the common region of Env from wild-type US4 (SEQ ID NO:69).

Figure 57 depicts the nucleotide sequence of the common region of Env from wild-type SF162 (SEQ ID NO:70).

10 Figure 58 depicts the nucleotide sequence of synthetic sequences corresponding to the common region of Env from US4 (SEQ ID NO:71).

Figure 59 depicts the nucleotide sequence of synthetic sequences corresponding to the common region of Env from SF162 (SEQ ID NO:72).

15 Figure 60 presents a schematic representation of an Env polypeptide purification strategy.

Figure 61 depicts the nucleotide sequence of the bicistronic construct designated gp160.modUS4.Gag.modSF2 (SEQ ID NO:73).

20 Figure 62 depicts the nucleotide sequence of the bicistronic construct designated gp160.modSF162.Gag.modSF2 (SEQ ID NO:74).

Figure 63 depicts the nucleotide sequence of the bicistronic construct designated gp160.modUS4.-delV1/V2.Gag.modSF2 (SEQ ID NO:75).

25 Figure 64 depicts the nucleotide sequence of the bicistronic construct designated gp160.modSF162.delV2.Gag.modSF2 (SEQ ID NO:76).

30 Figures 65A-65F show micrographs of 293T cells transfected with the following polypeptide encoding sequences: Figure 65A, gag.modSF2; Figure 65B, gp160.modUS4; Figure 65C, gp160.modUS4.delV1/V2.gag.modSF2 (bicistronic Env and Gag); Figures 65D and 65E, gp160.modUS4.delV1/V2 and

gag.modSF2; and Figure 65F, gp120.modSF162.delV2 and gag.modSF2.

Figures 66A and 66B present alignments of selected modified coding sequences of the present invention including a common region defined for each group of synthetic *Env* expression cassettes. Figure 66A presents alignments of modified SF162 sequences. Figure 66B presents alignments of modified US4 sequences. The SEQ ID NOs for these sequences are presented in Tables 1A and 1B.

Figure 67 shows the ELISA titers (binding antibodies) obtained in two rhesus macaques (H445, lines with solid black dots; and J408, lines with open squares). The y-axis is the end-point gp140 ELISA titers and the x-axis shows weeks post-immunization. The dashed lines at 0, 4, and 8 weeks represent DNA immunizations. The alternating dash/dotted line at 27 weeks indicates a DNA plus protein boost immunization.

Figure 68 (SEQ ID NO:77) depicts the wild-type nucleotide sequence of Gag reverse transcriptase from SF2.

Figure 69 (SEQ ID NO:78) depicts the nucleotide sequence of the construct designated GP1.

Figure 70 (SEQ ID NO:79) depicts the nucleotide sequence of the construct designated GP2.

Figure 71 (SEQ ID NO:80) depicts the nucleotide sequence of the construct designated FS(+).protinact.RTopt.YM. FS(+) indicates that there is a frameshift in the GagPol coding sequence.

Figure 72 (SEQ ID NO:81) depicts the nucleotide sequence of the construct designated FS(+).protinact.RTopt.YMWM.

Figure 73 (SEQ ID NO:82) depicts the nucleotide sequence of the construct designated FS(-

).protmod.RTopt.YM. FS(-) indicates that there is no frameshift in the GagPol coding sequence.

Figure 74 (SEQ ID NO:83) depicts the nucleotide sequence of the construct designated

5 FS(-).protmod.RTopt.YMWM.

Figure 75 (SEQ ID NO:84) depicts the nucleotide sequence of the construct designated FS(-).protmod.RTopt(+).

10 Figure 76 (SEQ ID NO:85) depicts the nucleotide sequence of wild type Tat from isolate SF162.

Figure 77 (SEQ ID NO:86) depicts the amino acid sequence of the tat polypeptide.

15 Figure 78 (SEQ ID NO:87) depicts the nucleotide sequence of a synthetic Tat construct designated Tat.SF162.opt.

20 Figure 79 (SEQ ID NO:88) depicts the nucleotide sequence of a synthetic Tat construct designated tat.cys22.sf162.opt. The construct encodes a tat polypeptide in which the cystein residue at position 22 of the wild type Tat polypeptide is replaced by a glycine residue.

Figures 80A to 80E are an alignment of the nucleotide sequences of the constructs designated Gag.mod.SF2, GP1 (SEQ ID NO:78), and GP2 (SEQ ID NO:79).

25 Figure 81 (SEQ ID NO:89) depicts the nucleotide sequence of the construct designated tataminoSF162.opt, which encodes the amino terminus of that tat protein. The codon encoding the cystein-22 residue is underlined.

30 Figure 82 (SEQ ID NO:90) depicts the amino acid sequence of the polypeptide encoded by the construct designated tat.cys22.SF162.opt (SEQ ID NO:88).

DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.); and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds., 1986, Blackwell Scientific Publications); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Short Protocols in Molecular Biology*, 4th ed. (Ausubel et al. eds., 1999, John Wiley & Sons); *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream et al., eds., 1998, Academic Press); *PCR (Introduction to Biotechniques Series)*, 2nd ed. (Newton & Graham eds., 1997, Springer Verlag).

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise. Thus, for example, reference to "an antigen" includes a mixture of two or more such agents.

1. DEFINITIONS

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

"Synthetic" sequences, as used herein, refers to Env-, tat- or Gag-encoding polynucleotides whose expression has been optimized as described herein, for example, by codon substitution, deletions, replacements and/or inactivation of inhibitory sequences. "Wild-type"

or "native" sequences, as used herein, refers to polypeptide encoding sequences that are essentially as they are found in nature, e.g., Gag encoding sequences as found in the isolate HIV-1SF2 or Env encoding sequences as found in the isolates HIV-1SF162 or HIV1US4.

As used herein, the term "virus-like particle" or "VLP" refers to a nonreplicating, viral shell, derived from any of several viruses discussed further below. VLPs are generally composed of one or more viral proteins, such as, but not limited to those proteins referred to as capsid, coat, shell, surface and/or envelope proteins, or particle-forming polypeptides derived from these proteins. VLPs can form spontaneously upon recombinant expression of the protein in an appropriate expression system. Methods for producing particular VLPs are known in the art and discussed more fully below. The presence of VLPs following recombinant expression of viral proteins can be detected using conventional techniques known in the art, such as by electron microscopy, biophysical characterization, and the like. See, e.g., Baker et al., *Biophys. J.* (1991) 60:1445-1456; Hagensee et al., *J. Virol.* (1994) 68:4503-4505. For example, VLPs can be isolated by density gradient centrifugation and/or identified by characteristic density banding (e.g., Example 7). Alternatively, cryoelectron microscopy can be performed on vitrified aqueous samples of the VLP preparation in question, and images recorded under appropriate exposure conditions.

By "particle-forming polypeptide" derived from a particular viral protein is meant a full-length or near full-length viral protein, as well as a fragment thereof, or a viral protein with internal deletions, which has the ability to form VLPs under conditions that favor VLP

formation. Accordingly, the polypeptide may comprise the full-length sequence, fragments, truncated and partial sequences, as well as analogs and precursor forms of the reference molecule. The term therefore intends

5 deletions, additions and substitutions to the sequence, so long as the polypeptide retains the ability to form a VLP. Thus, the term includes natural variations of the specified polypeptide since variations in coat proteins often occur between viral isolates. The term also

10 includes deletions, additions and substitutions that do not naturally occur in the reference protein, so long as the protein retains the ability to form a VLP. Preferred substitutions are those which are conservative in nature, i.e., those substitutions that take place within a family

15 of amino acids that are related in their side chains. Specifically, amino acids are generally divided into four families: (1) acidic -- aspartate and glutamate; (2) basic -- lysine, arginine, histidine; (3) non-polar -- alanine, valine, leucine, isoleucine, proline,

20 phenylalanine, methionine, tryptophan; and (4) uncharged polar -- glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids.

25 An "antigen" refers to a molecule containing one or more epitopes (either linear, conformational or both) that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term is used interchangeably with the term "immunogen."

30 Normally, a B-cell epitope will include at least about 5 amino acids but can be as small as 3-4 amino acids. A T-cell epitope, such as a CTL epitope, will include at least about 7-9 amino acids, and a helper T-cell epitope at least about 12-20 amino acids. Normally, an epitope

will include between about 7 and 15 amino acids, such as, 9, 10, 12 or 15 amino acids. The term "antigen" denotes both subunit antigens, (i.e., antigens which are separate and discrete from a whole organism with which the antigen is associated in nature), as well as, killed, attenuated or inactivated bacteria, viruses, fungi, parasites or other microbes. Antibodies such as anti-idiotypic antibodies, or fragments thereof, and synthetic peptide mimotopes, which can mimic an antigen or antigenic determinant, are also captured under the definition of antigen as used herein. Similarly, an oligonucleotide or polynucleotide which expresses an antigen or antigenic determinant *in vivo*, such as in gene therapy and DNA immunization applications, is also included in the definition of antigen herein.

For purposes of the present invention, antigens can be derived from any of several known viruses, bacteria, parasites and fungi, as described more fully below. The term also intends any of the various tumor antigens. Furthermore, for purposes of the present invention, an "antigen" refers to a protein which includes modifications, such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, so long as the protein maintains the ability to elicit an immunological response, as defined herein. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the antigens.

An "immunological response" to an antigen or composition is the development in a subject of a humoral and/or a cellular immune response to an antigen present in the composition of interest. For purposes of the present invention, a "humoral immune response" refers to

an immune response mediated by antibody molecules, while a "cellular immune response" is one mediated by T-lymphocytes and/or other white blood cells. One important aspect of cellular immunity involves an antigen-specific response by cytolytic T-cells ("CTL"s). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the destruction of intracellular microbes, or the lysis of cells infected with such microbes. Another aspect of cellular immunity involves an antigen-specific response by helper T-cells. Helper T-cells act to help stimulate the function, and focus the activity of, nonspecific effector cells against cells displaying peptide antigens in association with MHC molecules on their surface. A "cellular immune response" also refers to the production of cytokines, chemokines and other such molecules produced by activated T-cells and/or other white blood cells, including those derived from CD4+ and CD8+ T-cells.

A composition or vaccine that elicits a cellular immune response may serve to sensitize a vertebrate subject by the presentation of antigen in association with MHC molecules at the cell surface. The cell-mediated immune response is directed at, or near, cells presenting antigen at their surface. In addition, antigen-specific T-lymphocytes can be generated to allow for the future protection of an immunized host.

The ability of a particular antigen to stimulate a cell-mediated immunological response may be determined by a number of assays, such as by lymphoproliferation (lymphocyte activation) assays, CTL cytotoxic cell assays, or by assaying for T-lymphocytes specific for the

antigen in a sensitized subject. Such assays are well known in the art. See, e.g., Erickson et al., *J.*

Immunol. (1993) 151:4189-4199; Doe et al., *Eur. J.*

Immunol. (1994) 24:2369-2376. Recent methods of

5 measuring cell-mediated immune response include measurement of intracellular cytokines or cytokine secretion by T-cell populations, or by measurement of epitope specific T-cells (e.g., by the tetramer technique) (reviewed by McMichael, A.J., and O'Callaghan, C.A., *J. Exp. Med.* 187(9)1367-1371, 1998; Mcheyzer-Williams, M.G., et al, *Immunol. Rev.* 150:5-21, 1996; Lalvani, A., et al, *J. Exp. Med.* 186:859-865, 1997).

Thus, an immunological response as used herein may be one which stimulates the production of CTLs, and/or
15 the production or activation of helper T- cells. The antigen of interest may also elicit an antibody-mediated immune response. Hence, an immunological response may include one or more of the following effects: the production of antibodies by B-cells; and/or the
20 activation of suppressor T-cells and/or $\gamma\delta$ T-cells directed specifically to an antigen or antigens present in the composition or vaccine of interest. These responses may serve to neutralize infectivity, and/or mediate antibody-complement, or antibody dependent cell
25 cytotoxicity (ADCC) to provide protection to an immunized host. Such responses can be determined using standard immunoassays and neutralization assays, well known in the art.

An "immunogenic composition" is a composition that
30 comprises an antigenic molecule where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigenic molecule of interest.

By "subunit vaccine" is meant a vaccine composition which includes one or more selected antigens but not all antigens, derived from or homologous to, an antigen from a pathogen of interest such as from a virus, bacterium, parasite or fungus. Such a composition is substantially free of intact pathogen cells or pathogenic particles, or the lysate of such cells or particles. Thus, a "subunit vaccine" can be prepared from at least partially purified (preferably substantially purified) immunogenic polypeptides from the pathogen, or analogs thereof. The method of obtaining an antigen included in the subunit vaccine can thus include standard purification techniques, recombinant production, or synthetic production.

"Substantially purified" generally refers to isolation of a substance (compound, polynucleotide, protein, polypeptide, polypeptide composition) such that the substance comprises the majority percent of the sample in which it resides. Typically in a sample a substantially purified component comprises 50%, preferably 80%-85%, more preferably 90-95% of the sample. Techniques for purifying polynucleotides and polypeptides of interest are well-known in the art and include, for example, ion-exchange chromatography, affinity chromatography and sedimentation according to density.

A "coding sequence" or a sequence which "encodes" a selected polypeptide, is a nucleic acid molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences (or "control elements"). The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but

is not limited to, cDNA from viral, procaryotic or eucaryotic mRNA, genomic DNA sequences from viral or procaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence may be located 3' to the coding sequence.

Typical "control elements", include, but are not limited to, transcription promoters, transcription enhancer elements, transcription termination signals, polyadenylation sequences (located 3' to the translation stop codon), sequences for optimization of initiation of translation (located 5' to the coding sequence), and translation termination sequences, see e.g., McCaughan et al. (1995) *PNAS USA* 92:5431-5435; Kochetov et al (1998) *FEBS Letts.* 440:351-355.

A "nucleic acid" molecule can include, but is not limited to, procaryotic sequences, eucaryotic mRNA, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA.

"Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, a given promoter operably linked to a coding sequence is capable of effecting the expression of the coding sequence when the proper enzymes are present. The promoter need not be contiguous with the coding sequence, so long as it functions to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between the promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

"Recombinant" as used herein to describe a nucleic acid molecule means a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of the polynucleotide with which it is associated in nature; and/or (2) is linked to a polynucleotide other than that to which it is linked in nature. The term "recombinant" as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide. "Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting procaryotic microorganisms or eucaryotic cell lines cultured as unicellular entities, are used interchangeably, and refer to cells which can be, or have been, used as recipients for recombinant vectors or other transfer DNA, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation. Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide sequence encoding a desired peptide, are included in the progeny intended by this definition, and are covered by the above terms.

Techniques for determining amino acid sequence "similarity" are well known in the art. In general, "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then

can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively.

Two or more polynucleotide sequences can be compared by determining their "percent identity." Two or more amino acid sequences likewise can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or peptide sequences, is generally described as the number of exact matches between two aligned sequences divided by the length of the shorter sequence and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981). This algorithm can be extended to use with peptide sequences using the scoring matrix developed by Dayhoff, *Atlas of Protein Sequences and Structure*, M.O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, *Nucl. Acids Res.* 14(6):6745-6763 (1986). An implementation of this algorithm for nucleic acid and peptide sequences is provided by the Genetics Computer Group (Madison, WI) in their BestFit utility application. The default parameters for this method are

described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, WI). Other equally suitable programs for calculating the percent identity or
5 similarity between sequences are generally known in the art.

For example, percent identity of a particular nucleotide sequence to a reference sequence can be determined using the homology algorithm of Smith and
10 Waterman with a default scoring table and a gap penalty of six nucleotide positions. Another method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh,
15 developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages, the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap
20 extension penalty of one, and a gap of six). From the data generated, the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, such as the alignment program
25 BLAST, which can also be used with default parameters. For example, BLASTN and BLASTP can be used with the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by =
30 HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at

the following internet address:

<http://www.ncbi.nlm.gov/cgi-bin/BLAST>.

One of skill in the art can readily determine the proper search parameters to use for a given sequence in the above programs. For example, the search parameters may vary based on the size of the sequence in question. Thus, for example, a representative embodiment of the present invention would include an isolated

polynucleotide having X contiguous nucleotides, wherein

(i) the X contiguous nucleotides have at least about 50% identity to Y contiguous nucleotides derived from any of the sequences described herein, (ii) X equals Y, and (iii) X is greater than or equal to 6 nucleotides and up to 5000 nucleotides, preferably greater than or equal to 8 nucleotides and up to 5000 nucleotides, more preferably 10-12 nucleotides and up to 5000 nucleotides, and even more preferably 15-20 nucleotides, up to the number of nucleotides present in the full-length sequences described herein (e.g., see the Sequence Listing and claims), including all integer values falling within the above-described ranges.

The synthetic expression cassettes (and purified polynucleotides) of the present invention include related polynucleotide sequences having about 80% to 100%, greater than 80-85%, preferably greater than 90-92%, more preferably greater than 95%, and most preferably greater than 98% sequence (including all integer values falling within these described ranges) identity to the synthetic expression cassette sequences disclosed herein (for example, to the sequences presented in Tables 1A and 1B) when the sequences of the present invention are used as the query sequence.

Two nucleic acid fragments are considered to "selectively hybridize" as described herein. The degree of sequence identity between two nucleic acid molecules affects the efficiency and strength of hybridization events between such molecules. A partially identical nucleic acid sequence will at least partially inhibit a completely identical sequence from hybridizing to a target molecule. Inhibition of hybridization of the completely identical sequence can be assessed using hybridization assays that are well known in the art (e.g., Southern blot, Northern blot, solution hybridization, or the like, see Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, (1989) Cold Spring Harbor, N.Y.). Such assays can be conducted using varying degrees of selectivity, for example, using conditions varying from low to high stringency. If conditions of low stringency are employed, the absence of non-specific binding can be assessed using a secondary probe that lacks even a partial degree of sequence identity (for example, a probe having less than about 30% sequence identity with the target molecule), such that, in the absence of non-specific binding events, the secondary probe will not hybridize to the target.

When utilizing a hybridization-based detection system, a nucleic acid probe is chosen that is complementary to a target nucleic acid sequence, and then by selection of appropriate conditions the probe and the target sequence "selectively hybridize," or bind, to each other to form a hybrid molecule. A nucleic acid molecule that is capable of hybridizing selectively to a target sequence under "moderately stringent" typically

hybridizes under conditions that allow detection of a target nucleic acid sequence of at least about 10-14 nucleotides in length having at least approximately 70% sequence identity with the sequence of the selected nucleic acid probe. Stringent hybridization conditions typically allow detection of target nucleic acid sequences of at least about 10-14 nucleotides in length having a sequence identity of greater than about 90-95% with the sequence of the selected nucleic acid probe.

Hybridization conditions useful for probe/target hybridization where the probe and target have a specific degree of sequence identity, can be determined as is known in the art (see, for example, Nucleic Acid Hybridization: A Practical Approach, editors B.D. Hames and S.J. Higgins, (1985) Oxford; Washington, DC; IRL Press).

With respect to stringency conditions for hybridization, it is well known in the art that numerous equivalent conditions can be employed to establish a particular stringency by varying, for example, the following factors: the length and nature of probe and target sequences, base composition of the various sequences, concentrations of salts and other hybridization solution components, the presence or absence of blocking agents in the hybridization solutions (e.g., formamide, dextran sulfate, and polyethylene glycol), hybridization reaction temperature and time parameters, as well as, varying wash conditions. The selection of a particular set of hybridization conditions is selected following standard methods in the art (see, for example, Sambrook, et al., Molecular Cloning: A

Laboratory Manual, Second Edition, (1989) Cold Spring Harbor, N.Y.).

5 A first polynucleotide is "derived from" second polynucleotide if it has the same or substantially the same basepair sequence as a region of the second polynucleotide, its cDNA, complements thereof, or if it displays sequence identity as described above.

10 A first polypeptide is "derived from" a second polypeptide if it is (i) encoded by a first polynucleotide derived from a second polynucleotide, or (ii) displays sequence identity to the second polypeptides as described above.

15 Generally, a viral polypeptide is "derived from" a particular polypeptide of a virus (viral polypeptide) if it is (i) encoded by an open reading frame of a polynucleotide of that virus (viral polynucleotide), or (ii) displays sequence identity to polypeptides of that virus as described above.

20 "Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 to 5 amino acids, more preferably at least 8 to 10 amino acids, and even more preferably at least 15 to 20 amino acids from a polypeptide encoded by
25 the nucleic acid sequence. Also encompassed are polypeptide sequences which are immunologically identifiable with a polypeptide encoded by the sequence.

30 "Purified polynucleotide" refers to a polynucleotide of interest or fragment thereof which is essentially free, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for

example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density.

By "nucleic acid immunization" is meant the introduction of a nucleic acid molecule encoding one or more selected antigens into a host cell, for the *in vivo* expression of an antigen, antigens, an epitope, or epitopes. The nucleic acid molecule can be introduced directly into a recipient subject, such as by injection, inhalation, oral, intranasal and mucosal administration, or the like, or can be introduced *ex vivo*, into cells which have been removed from the host. In the latter case, the transformed cells are reintroduced into the subject where an immune response can be mounted against the antigen encoded by the nucleic acid molecule.

"Gene transfer" or "gene delivery" refers to methods or systems for reliably inserting DNA or RNA of interest into a host cell. Such methods can result in transient expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (e.g., episomes), or integration of transferred genetic material into the genomic DNA of host cells. Gene delivery expression vectors include, but are not limited to, vectors derived from bacterial plasmid vectors, viral vectors, non-viral vectors, alphaviruses, pox viruses and vaccinia viruses. When used for immunization, such gene delivery expression vectors may be referred to as vaccines or vaccine vectors.

"T lymphocytes" or "T cells" are non-antibody producing lymphocytes that constitute a part of the cell-mediated arm of the immune system. T cells arise from immature lymphocytes that migrate from the bone marrow to

the thymus, where they undergo a maturation process under the direction of thymic hormones. Here, the mature lymphocytes rapidly divide increasing to very large numbers. The maturing T cells become immunocompetent
5 based on their ability to recognize and bind a specific antigen. Activation of immunocompetent T cells is triggered when an antigen binds to the lymphocyte's surface receptors.

The term "transfection" is used to refer to the
10 uptake of foreign DNA by a cell. A cell has been "transfected" when exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. See, e.g., Graham et al. (1973) *Virology*, 52:456, Sambrook et al.
15 (1989) *Molecular Cloning, a laboratory manual*, Cold Spring Harbor Laboratories, New York, Davis et al. (1986) *Basic Methods in Molecular Biology*, Elsevier, and Chu et al. (1981) *Gene* 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into
20 suitable host cells. The term refers to both stable and transient uptake of the genetic material, and includes uptake of peptide- or antibody-linked DNAs.

A "vector" is capable of transferring gene sequences to target cells (e.g., bacterial plasmid vectors, viral
25 vectors, non-viral vectors, particulate carriers, and liposomes). Typically, "vector construct," "expression vector," and "gene transfer vector," mean any nucleic acid construct capable of directing the expression of a gene of interest and which can transfer gene sequences to
30 target cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

Transfer of a "suicide gene" (e.g., a drug-susceptibility gene) to a target cell renders the cell sensitive to compounds or compositions that are

relatively nontoxic to normal cells. Moolten, F.L. (1994) *Cancer Gene Ther.* 1:279-287. Examples of suicide genes are thymidine kinase of herpes simplex virus (HSV-tk), cytochrome P450 (Manome et al. (1996) *Gene Therapy* 3:513-520), human deoxycytidine kinase (Manome et al. (1996) *Nature Medicine* 2(5):567-573) and the bacterial enzyme cytosine deaminase (Dong et al. (1996) *Human Gene Therapy* 7:713-720). Cells which express these genes are rendered sensitive to the effects of the relatively nontoxic prodrugs ganciclovir (HSV-tk), cyclophosphamide (cytochrome P450 2B1), cytosine arabinoside (human deoxycytidine kinase) or 5-fluorocytosine (bacterial cytosine deaminase). Culver et al. (1992) *Science* 256:1550-1552, Huber et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:8302-8306.

A "selectable marker" or "reporter marker" refers to a nucleotide sequence included in a gene transfer vector that has no therapeutic activity, but rather is included to allow for simpler preparation, manufacturing, characterization or testing of the gene transfer vector.

A "specific binding agent" refers to a member of a specific binding pair of molecules wherein one of the molecules specifically binds to the second molecule through chemical and/or physical means. One example of a specific binding agent is an antibody directed against a selected antigen.

By "subject" is meant any member of the subphylum chordata, including, without limitation, humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such

as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like. The term does not denote a particular age. Thus, both adult and newborn individuals are intended to be covered. The system described above
5 is intended for use in any of the above vertebrate species, since the immune systems of all of these vertebrates operate similarly.

By "pharmaceutically acceptable" or "pharmacologically acceptable" is meant a material which
10 is not biologically or otherwise undesirable, i.e., the material may be administered to an individual in a formulation or composition without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the
15 composition in which it is contained.

By "physiological pH" or a "pH in the physiological range" is meant a pH in the range of approximately 7.2 to 8.0 inclusive, more typically in the range of approximately 7.2 to 7.6 inclusive.

20 As used herein, "treatment" refers to any of (i) the prevention of infection or reinfection, as in a traditional vaccine, (ii) the reduction or elimination of symptoms, and (iii) the substantial or complete elimination of the pathogen in question. Treatment may
25 be effected prophylactically (prior to infection) or therapeutically (following infection).

"Lentiviral vector", and "recombinant lentiviral vector" are derived from the subset of retroviral vectors known as lentiviruses. Lentiviral vectors refer to a
30 nucleic acid construct which carries, and within certain embodiments, is capable of directing the expression of a nucleic acid molecule of interest. The lentiviral vector includes at least one transcriptional promoter/enhancer or locus defining element(s), or other elements which

control gene expression by other means such as alternate splicing, nuclear RNA export, post-translational modification of messenger, or post-transcriptional modification of protein. Such vector constructs must
5 also include a packaging signal, long terminal repeats (LTRS) or portion thereof, and positive and negative strand primer binding sites appropriate to the lentiviral vector used (if these are not already present in the retroviral vector). Optionally, the recombinant
10 lentiviral vector may also include a signal which directs polyadenylation, selectable markers such as Neo, TK, hygromycin, phleomycin, histidinol, or DHFR, as well as one or more restriction sites and a translation termination sequence. By way of example, such vectors
15 typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis, and a 3'LTR or a portion thereof.

"Lentiviral vector particle" as utilized within the present invention refers to a lentivirus which carries at
20 least one gene of interest. The retrovirus may also contain a selectable marker. The recombinant lentivirus is capable of reverse transcribing its genetic material (RNA) into DNA and incorporating this genetic material into a host cell's DNA upon infection. Lentiviral vector
25 particles may have a lentiviral envelope, a non-lentiviral envelope (e.g., an amphi or VSV-G envelope), or a chimeric envelope.

"Nucleic acid expression vector" or "Expression cassette" refers to an assembly which is capable of
30 directing the expression of a sequence or gene of interest. The nucleic acid expression vector includes a promoter which is operably linked to the sequences or gene(s) of interest. Other control elements may be present as well. Expression cassettes described herein

may be contained within a plasmid construct. In addition to the components of the expression cassette, the plasmid construct may also include a bacterial origin of replication, one or more selectable markers, a signal
5 which allows the plasmid construct to exist as single-stranded DNA (e.g., a M13 origin of replication), a multiple cloning site, and a "mammalian" origin of replication (e.g., a SV40 or adenovirus origin of replication).

10 "Packaging cell" refers to a cell which contains those elements necessary for production of infectious recombinant retrovirus (e.g., lentivirus) which are lacking in a recombinant retroviral vector. Typically, such packaging cells contain one or more expression
15 cassettes which are capable of expressing proteins which encode *Gag*, *pol* and *env* proteins.

"Producer cell" or "vector producing cell" refers to a cell which contains all elements necessary for production of recombinant retroviral vector particles.

20

2. MODES OF CARRYING OUT THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such
25 may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar
30 or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

2.1 SYNTHETIC EXPRESSION CASSETTES

2.1.1 MODIFICATION OF HIV-1 GAG NUCLEIC ACID CODING SEQUENCES

One aspect of the present invention is the generation of HIV-1 Gag protein coding sequences, and related sequences, having improved expression relative to the corresponding wild-type sequence. An exemplary embodiment of the present invention is illustrated herein modifying the Gag protein wild-type sequences obtained from the HIV-1SF2 strain (SEQ ID NO:1; Sanchez-Pescador, R., et al., *Science* 227(4686): 484-492, 1985; Luciw, P.A., et al. U.S. Patent No. 5,156,949, issued October 20, 1992; Luciw, P.A., et al., U.S. Patent No. 5,688,688, November 18, 1997). Gag sequence obtained from other HIV variants may be manipulated in similar fashion following the teachings of the present specification. Such other variants include, but are not limited to, Gag protein encoding sequences obtained from the isolates HIV_{IIIB}, HIV_{SF2}, HIV-1_{SF162}, HIV-1_{SF170}, HIV_{LAV}, HIV_{LA1}, HIV_{MN}, HIV-1_{CM235}, HIV-1_{US4}, other HIV-1 strains from diverse subtypes (e.g., subtypes, A through G, and O), HIV-2 strains and diverse subtypes (e.g., HIV-2_{UC1} and HIV-2_{UC2}), and simian immunodeficiency virus (SIV). (See, e.g., *Virology*, 3rd Edition (W.K. Joklik ed. 1988); *Fundamental Virology*, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991); *Virology*, 3rd Edition (Fields, BN, DM Knipe, PM Howley, Editors, 1996, Lippincott-Raven, Philadelphia, PA; for a description of these and other related viruses).

First, the HIV-1 codon usage pattern was modified so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes (Example 1). The HIV codon usage reflects a high content of the nucleotides A or T of the codon-triplet.

The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Gag coding sequences were modified to be comparable to codon usage found in highly expressed human genes. In Figure 11 (Example 1), the percent A-T content of cDNA sequences corresponding to the mRNA for a known unstable mRNA and a known stable mRNA are compared to the percent A-T content of native HIV-1SF2 Gag cDNA and to the synthetic Gag cDNA sequence of the present invention. Experiments performed in support of the present invention showed that the synthetic Gag sequences were capable of higher level of protein production (see the Examples) relative to the native Gag sequences. The data in Figure 11 suggest that one reason for this increased production is increased stability of the mRNA corresponding to the synthetic Gag coding sequences versus the mRNA corresponding to the native Gag coding sequences.

Second, there are inhibitory (or instability) elements (INS) located within the coding sequences of the Gag coding sequences (Example 1). The RRE is a secondary RNA structure that interacts with the HIV encoded Rev-protein to overcome the expression down-regulating effects of the INS. To overcome the post-transcriptional activating mechanisms of RRE and Rev, the instability elements were inactivated by introducing multiple point mutations that did not alter the reading frame of the encoded proteins. Figure 1 shows the original SF2 Gag sequence, the location of the INS sequences, and the modifications made to the INS sequences to reduce their effects. The resulting modified coding sequences are

presented as a synthetic Gag expression cassette (SEQ ID NO:4).

Modification of the Gag polypeptide coding sequences resulted in improved expression relative to the wild-type coding sequences in a number of mammalian cell lines (as well as other types of cell lines, including, but not limited to, insect cells). Further, expression of the sequences resulted in production of virus-like particles (VLPs) by these cell lines (see below). Similar Gag polypeptide coding sequences can be obtained from a variety of isolates (families, sub-types, strains, etc.) including, but not limited to such other variants include, but are not limited to, Gag polypeptide encoding sequences obtained from the isolates HIV_{IIIB}, HIV_{SF2}, HIV-1_{SF162}, HIV-1_{SF170}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}, HIV-1_{CM235}, HIV-1_{USA}, other HIV-1 strains from diverse subtypes (e.g., subtypes, A through G, and O), HIV-2 strains and diverse subtypes (e.g., HIV-2_{UC1} and HIV-2_{UC2}), and simian immunodeficiency virus (SIV). (See, e.g., Virology, 3rd Edition (W.K. Joklik ed. 1988); *Fundamental Virology*, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991; *Virology*, 3rd Edition (Fields, BN, DM Knipe, PM Howley, Editors, 1996, Lippincott-Raven, Philadelphia, PA). Gag polypeptide encoding sequences derived from these variants can be optimized and tested for improved expression in mammals by following the teachings of the present specification (see the Examples, in particular Example 1).

2.1.2 FURTHER MODIFICATION OF SEQUENCES INCLUDING HIV-1 GAG NUCLEIC ACID CODING SEQUENCES

Experiments performed in support of the present invention have shown that similar modifications of HIV-1 Gag-protease, Gag-reverse transcriptase and Gag-polymerase sequences also result in improved expression

of the polyproteins, as well as, the production of VLPs formed by polypeptides produced from such modified coding sequences.

For the Gag-protease sequence (wild type, SEQ ID NO:2; modified, SEQ ID NOs:5, 78, 79), the changes in codon usage were restricted to the regions upstream of the -1 frameshift (Figure 2). Further, inhibitory (or instability) elements (INS) located within the coding sequences of the Gag-protease polypeptide coding sequence were altered as well (indicated in Figure 2). Exemplary constructs (which include the -1 frameshift) encoding modified Gag-protease sequences include those shown in SEQ ID NOs:78 and 79 (Figures 69 and 70). These are: GP1 (SEQ ID NO:78) in which the protease region was also codon optimized and INS inactivated and GP2 (SEQ ID NO:79), in which the protease region was only subjected to INS inactivation.

For other Gag-containing sequences, for example the Gag-polymerase sequence (wild type, SEQ ID NO:3; modified, SEQ ID NO:6) or Gag-reverse transcriptase (wild type, SEQ ID NO:77; modified SEQ ID NOs:80-84), the changes in codon usage are similar to those for the Gag-protease sequence. Those expression cassettes which contain a frameshift in the GagPol coding sequence are designated "FS(+)" (SEQ ID NOs:80 and 81, Figures 71 and 72) while the designation "FS(-)" (SEQ ID Nos: 82, 83 and 84, Figures 73, 74 and 75) indicates that there is no frameshift utilized in this coding sequence.

In addition to polyproteins containing HIV-related sequences, the various Gag-, Gag-prot, Gag-pol, Gag-reverse transcriptase encoding sequences of the present invention can be fused to other polypeptides (creating chimeric polypeptides) for which an immunogenic response is desired. An example of such a chimeric protein is the

joining of the improved expression Gag encoding sequences to the Hepatitis C Virus (HCV) core protein. In this case, the HCV-core encoding sequences were placed in-frame with the HIV-Gag encoding sequences, resulting in the Gag/HCV-core encoding sequence presented as SEQ ID NO:7 (wild type sequence presented as SEQ ID NO:8).

Further sequences useful in the practice of the present invention include, but are not limited to, sequences encoding viral epitopes/antigens (including but not limited to, HCV antigens (e.g., E1, E2; Houghton, M., et al., U.S. Patent No. 5,714,596, issued February 3, 1998; Houghton, M., et al., U.S. Patent No. 5,712,088, issued January 27, 1998; Houghton, M., et al., U.S. Patent No. 5,683,864, issued November 4, 1997; Weiner, A.J., et al., U.S. Patent No. 5,728,520, issued March 17, 1998; Weiner, A.J., et al., U.S. Patent No. 5,766,845, issued June 16, 1998; Weiner, A.J., et al., U.S. Patent No. 5,670,152, issued September 23, 1997), HIV antigens (e.g., derived from *nef*, *tat*, *rev*, *vpu*, *vif*, *vpr* and/or *env*); and sequences encoding tumor antigens/epitopes. Additional sequences are described below. Also, variations on the orientation of the Gag and other coding sequences, relative to each other, are also described below.

Gag, Gag-protease, Gag-reverse transcriptase and/or Gag-polymerase polypeptide coding sequences can be obtained from any HIV isolates (different families, subtypes, and strains) including but not limited to the isolates HIV_{IIIB}, HIV_{SF2}, HIV_{SF162}, HIV_{US4}, HIV_{CM235}, HIV_{LAV}, HIV_{LA1}, HIV_{MN} (see, e.g., Myers et al. Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico (1992); Myers et al., *Human Retroviruses and Aids*, 1997, Los Alamos, New Mexico: Los Alamos National Laboratory). Synthetic expression cassettes can be generated using

such coding sequences as starting material by following the teachings of the present specification (e.g., see Example 1). Further, the synthetic expression cassettes of the present invention include related Gag polypeptide coding sequences having greater than 75%, preferably greater than 80-85%, more preferably greater than 90-95%, and most preferably greater than 98% sequence identity (or any integer value within these ranges) to the synthetic expression cassette sequences disclosed herein (for example, SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; and SEQ ID NO:20, the Gag Major Homology Region).

2.1.3 EXPRESSION OF SYNTHETIC SEQUENCES ENCODING HIV-1 GAG AND RELATED POLYPEPTIDES

Several synthetic Gag-encoding sequences (expression cassettes) of the present invention were cloned into a number of different expression vectors (Example 1) to evaluate levels of expression and production of VLPs. Two modified synthetic coding sequences are presented as a synthetic Gag expression cassette (SEQ ID NO:4) and a synthetic Gag-protease expression cassette (SEQ ID NOs:78 and 79). Other synthetic Gag-encoding proteins are presented, for example, as SEQ ID NOs:80 through 84. The synthetic DNA fragments for Gag-encoding polypeptides (e.g., Gag, Gag-protease, Gag-polymerase, Gag-reverse transcriptase) were cloned into expression vectors described in Example 1, including, a transient expression vector, CMV-promoter-based mammalian vectors, and a shuttle vector for use in baculovirus expression systems. Corresponding wild-type sequences were cloned into the same vectors.

These vectors were then transfected into a several different cell types, including a variety of mammalian

cell lines, (293, RD, COS-7, and CHO, cell lines available, for example, from the A.T.C.C.). The cell lines were cultured under appropriate conditions and the levels of p24 (Gag) expression in supernatants were
5 evaluated (Example 2). The results of these assays demonstrated that expression of synthetic Gag-encoding sequences were significantly higher than corresponding wild-type sequences (Example 2; Table 2).

Further, Western Blot analysis showed that cells
10 containing the synthetic Gag expression cassette produced the expected 55 kD (p55) protein at higher per-cell concentrations than cells containing the native expression cassette. The Gag p55 protein was seen in both cell lysates and supernatants. The levels of
15 production were significantly higher in cell supernatants for cells transfected with the synthetic Gag expression cassette of the present invention. Experiments performed in support of the present invention suggest that cells containing the synthetic Gag-prot expression cassettes
20 produced the expected Gag-prot protein at comparably higher per-cell concentrations than cells containing the wild-type expression cassette.

Fractionation of the supernatants from mammalian cells transfected with the synthetic Gag expression
25 cassette showed that it provides superior production of both p55 protein and VLPs, relative to the wild-type Gag sequences (Examples 6 and 7).

Efficient expression of these Gag-containing polypeptides in mammalian cell lines provides the
30 following benefits: the Gag polypeptides are free of baculovirus contaminants; production by established methods approved by the FDA; increased purity; greater yields (relative to native coding sequences); and a novel method of producing the Gag-containing polypeptides in

CHO or other mammalian cells which is not feasible in the absence of the increased expression obtained using the constructs of the present invention. Exemplary Mammalian cell lines include, but are not limited to, BHK, VERO, HT1080, 293, 293T, RD, COS-7, CHO, Jurkat, HUT, SUPT, C8166, MOLT4/clone8, MT-2, MT-4, H9, PM1, CEM, myeloma cells (e.g., SB20 cells) and CEMX174, such cell lines are available, for example, from the A.T.C.C.).

A synthetic Gag expression cassette of the present invention also demonstrated high levels of expression and VLP production when transfected into insect cells (Example 7). Further, in addition to a higher total protein yield, the final product from the synthetic p55-expressed Gag consistently contained lower amounts of contaminating baculovirus proteins than the final purified product from the native p55-expressed Gag.

Further, synthetic Gag expression cassettes of the present invention have also been introduced into yeast vectors which were transformed into and efficiently expressed by yeast cells (*Saccharomyces cerevisiae*; using vectors as described in Rosenberg, S. and Tekamp-Olson, P., U.S. Patent No. RE35,749, issued, March 17, 1998).

In addition to the mammalian and insect vectors described in the Examples, the synthetic expression cassettes of the present invention can be incorporated into a variety of expression vectors using selected expression control elements. Appropriate vectors and control elements for any given cell type can be selected by one having ordinary skill in the art in view of the teachings of the present specification and information known in the art about expression vectors.

For example, a synthetic Gag expression cassette can be inserted into a vector which includes control elements operably linked to the desired coding sequence, which

allow for the expression of the gene in a selected cell-type. For example, typical promoters for mammalian cell expression include the SV40 early promoter, a CMV promoter such as the CMV immediate early promoter (a CMV promoter can include intron A), RSV, HIV-LTR, the mouse mammary tumor virus LTR promoter (MMLV-LTR), FIV-LTR, the adenovirus major late promoter (Ad MLP), and the herpes simplex virus promoter, among others. Other nonviral promoters, such as a promoter derived from the murine metallothionein gene, will also find use for mammalian expression. Typically, transcription termination and polyadenylation sequences will also be present, located 3' to the translation stop codon. Preferably, a sequence for optimization of initiation of translation, located 5' to the coding sequence, is also present. Examples of transcription terminator/polyadenylation signals include those derived from SV40, as described in Sambrook, et al., *supra*, as well as a bovine growth hormone terminator sequence. Introns, containing splice donor and acceptor sites, may also be designed into the constructs for use with the present invention (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986).

Enhancer elements may also be used herein to increase expression levels of the mammalian constructs. Examples include the SV40 early gene enhancer, as described in Dijkema et al., *EMBO J.* (1985) 4:761, the enhancer/promoter derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus, as described in Gorman et al., *Proc. Natl. Acad. Sci. USA* (1982b) 79:6777 and elements derived from human CMV, as described in Boshart et al., *Cell* (1985) 41:521, such as elements included in the CMV intron A sequence (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986).

The desired synthetic Gag polypeptide encoding sequences can be cloned into any number of commercially available vectors to generate expression of the polypeptide in an appropriate host system. These systems include, but are not limited to, the following:

5 baculovirus expression {Reilly, P.R., et al., BACULOVIRUS EXPRESSION VECTORS: A LABORATORY MANUAL (1992); Beames, et al., *Biotechniques* 11:378 (1991); Pharmingen; Clontech, Palo Alto, CA}}, vaccinia expression {Earl, P. L., et al.,

10 "Expression of proteins in mammalian cells using vaccinia" In *Current Protocols in Molecular Biology* (F. M. Ausubel, et al. Eds.), Greene Publishing Associates & Wiley Interscience, New York (1991); Moss, B., et al., U.S. Patent Number 5,135,855, issued 4 August 1992},

15 expression in bacteria {Ausubel, F.M., et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, Inc., Media PA; Clontech}, expression in yeast {Rosenberg, S. and Tekamp-Olson, P., U.S. Patent No. RE35,749, issued, March 17, 1998; Shuster, J.R., U.S. Patent No. 5,629,203,

20 issued May 13, 1997; Gellissen, G., et al., *Antonie Van Leeuwenhoek*, 62(1-2):79-93 (1992); Romanos, M.A., et al., *Yeast* 8(6):423-488 (1992); Goeddel, D.V., *Methods in Enzymology* 185 (1990); Guthrie, C., and G.R. Fink, *Methods in Enzymology* 194 (1991)}, expression in

25 mammalian cells {Clontech; Gibco-BRL, Ground Island, NY; e.g., Chinese hamster ovary (CHO) cell lines (Haynes, J., et al., *Nuc. Acid. Res.* 11:687-706 (1983); 1983, Lau, Y.F., et al., *Mol. Cell. Biol.* 4:1469-1475 (1984); Kaufman, R. J., "Selection and coamplification of

30 heterologous genes in mammalian cells," in *Methods in Enzymology*, vol. 185, pp537-566. Academic Press, Inc., San Diego CA (1991)}, and expression in plant cells {plant cloning vectors, Clontech Laboratories, Inc., Palo Alto, CA, and Pharmacia LKB Biotechnology, Inc.,

Piscataway, NJ; Hood, E., et al., *J. Bacteriol.* 168:1291-1301 (1986); Nagel, R., et al., *FEMS Microbiol. Lett.* 67:325 (1990); An, et al., "Binary Vectors", and others in Plant Molecular Biology Manual A3:1-19 (1988);
5 Miki, B.L.A., et al., pp.249-265, and others in Plant DNA Infectious Agents (Hohn, T., et al., eds.) Springer-Verlag, Wien, Austria, (1987); *Plant Molecular Biology: Essential Techniques*, P.G. Jones and J.M. Sutton, New York, J. Wiley, 1997; Miglani, Gurbachan *Dictionary of*
10 *Plant Genetics and Molecular Biology*, New York, Food Products Press, 1998; Henry, R. J., *Practical Applications of Plant Molecular Biology*, New York, Chapman & Hall, 1997}.

Also included in the invention is an expression
15 vector, such as the CMV promoter-containing vectors described in Example 1, containing coding sequences and expression control elements which allow expression of the coding regions in a suitable host. The control elements generally include a promoter, translation initiation
20 codon, and translation and transcription termination sequences, and an insertion site for introducing the insert into the vector. Translational control elements have been reviewed by M. Kozak (e.g., Kozak, M., *Mamm. Genome* 7(8):563-574, 1996; Kozak, M., *Biochimie*
25 76(9):815-821, 1994; Kozak, M., *J Cell Biol* 108(2):229-241, 1989; Kozak, M., and Shatkin, A.J., *Methods Enzymol* 60:360-375, 1979).

Expression in yeast systems has the advantage of commercial production. Recombinant protein production by
30 vaccinia and CHO cell line have the advantage of being mammalian expression systems. Further, vaccinia virus expression has several advantages including the following: (i) its wide host range; (ii) faithful post-

transcriptional modification, processing, folding, transport, secretion, and assembly of recombinant proteins; (iii) high level expression of relatively soluble recombinant proteins; and (iv) a large capacity to accommodate foreign DNA.

The recombinantly expressed polypeptides from synthetic Gag-encoding expression cassettes are typically isolated from lysed cells or culture media. Purification can be carried out by methods known in the art including salt fractionation, ion exchange chromatography, gel filtration, size-exclusion chromatography, size-fractionation, and affinity chromatography.

Immunoaffinity chromatography can be employed using antibodies generated based on, for example, Gag antigens.

Advantages of expressing the Gag-containing proteins of the present invention using mammalian cells include, but are not limited to, the following: well-established protocols for scale-up production; the ability to produce VLPs; cell lines are suitable to meet good manufacturing process (GMP) standards; culture conditions for mammalian cells are known in the art.

2.1.4 MODIFICATION OF HIV-1 ENV NUCLEIC ACID CODING SEQUENCES

One aspect of the present invention is the generation of HIV-1 Env protein coding sequences, and related sequences, having improved expression relative to the corresponding wild-type sequence. Exemplary embodiments of the present invention are illustrated herein modifying the Env protein wild-type sequences obtained from the HIV-1 subtype B strains HIV-1US4 and HIV-1SF162 (Myers et al., Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico (1992); Myers et al., *Human Retroviruses and Aids*, 1997, Los Alamos,

New Mexico: Los Alamos National Laboratory). Env sequence obtained from other HIV variants may be manipulated in similar fashion following the teachings of the present specification. Such other variants include those
5 described above in Section 2.1.1 and on the World Wide Web (Internet), for example at <http://hiv-web.lanl.gov/cgi-bin/hivDB3/public/wdb/ssampublic> and <http://hiv-web.lanl.gov>.

First, the HIV-1 codon usage pattern was modified so
10 that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes (Example 1). The HIV codon usage reflects a high content of the nucleotides A or T of the codon-triplet. The effect of the HIV-1 codon usage is a high AT content
15 in the DNA sequence that results in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Env coding sequences were modified to be comparable to codon usage found in highly
20 expressed human genes. Experiments performed in support of the present invention showed that the synthetic Env sequences were capable of higher level of protein production (see the Examples) relative to the native Env sequences. One reason for this increased production may
25 be increased stability of the mRNA corresponding to the synthetic Env coding sequences versus the mRNA corresponding to the native Env coding sequences.

Modification of the Env polypeptide coding sequences resulted in improved expression relative to the wild-type
30 coding sequences in a number of mammalian cell lines. Similar Env polypeptide coding sequences can be obtained from a variety of isolates (families, sub-types, etc.). Env polypeptide encoding sequences derived from these variants can be optimized and tested for improved

expression in mammals by following the teachings of the present specification (see the Examples, in particular Example 2).

5 2.1.5 FURTHER MODIFICATION OF HIV-1 ENV NUCLEIC ACID
 CODING SEQUENCES

 In addition to proteins containing HIV-related sequences, the Env encoding sequences of the present invention can be fused to other polypeptides (creating
10 chimeric polypeptides). Also, variations on the orientation of the Env and other coding sequences, relative to each other, are contemplated. Further, the HIV protein encoding cassettes of the present invention can be co-expressed using one vector or multiple vectors.
15 In addition, the polyproteins can be operably linked to the same or different promoters.

 Env polypeptide coding sequences can be obtained from any HIV isolates (different families, subtypes, and strains) including but not limited to the isolates HIV_{IIIB},
20 HIV_{SF2}, HIV_{US4}, HIV_{CM235}, HIV_{SF162}, HIV_{LAV}, HIV_{LA1}, HIV_{MN}) (see, e.g., Myers et al., Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico (1992); Myers et al., *Human Retroviruses and Aids*, 1997, Los Alamos, New Mexico: Los Alamos National Laboratory). Synthetic
25 expression cassettes can be generated using such coding sequences as starting material by following the teachings of the present specification (e.g., see Example 1). Further, the synthetic expression cassettes (and purified polynucleotides) of the present invention include related
30 Env polypeptide coding sequences having greater than 90%, preferably greater than 92%, more preferably greater than 95%, and most preferably greater than 98% sequence identity to the synthetic expression cassette sequences disclosed herein (for example, SEQ ID NOs:71-72; and/or

the sequences presented in Tables 1A and 1B) when the sequences of the present invention are used as the query sequence.

5 2.1.6 EXPRESSION OF SYNTHETIC SEQUENCES ENCODING HIV-1
 ENV AND RELATED POLYPEPTIDES

Several synthetic Env-encoding sequences (expression cassettes) of the present invention were cloned into a number of different expression vectors (Example 1) to
10 evaluate levels of expression and production of Env polypeptide. A modified synthetic coding sequence is presented as synthetic Env expression cassettes (Example 1, e.g., Tables 1A and 1B). The synthetic DNA fragments for Env were cloned into eucaryotic expression vectors
15 described in Example 1 and in Section 2.1.3 above, including, a transient expression vector and CMV-promoter-based mammalian vectors. Corresponding wild-type sequences were cloned into the same vectors.

These vectors were then transfected into a several
20 different cell types, including a variety of mammalian cell lines, (293, RD, COS-7, and CHO, cell lines available, for example, from the A.T.C.C.). The cell lines were cultured under appropriate conditions and the levels of gp120, gp140 and gp160 Env expression in
25 supernatants were evaluated (Example 2). Env polypeptides include, but are not limited to, for example, native gp160, oligomeric gp140, monomeric gp120 as well as modified sequences of these polypeptides. The results of these assays demonstrated that expression of
30 synthetic Env encoding sequences were significantly higher than corresponding wild-type sequences (Example 2; Tables 3 and 4).

Further, Western Blot analysis showed that cells containing the synthetic Env expression cassette produced

the expected protein (gp120, gp140 or gp160) at higher per-cell concentrations than cells containing the native expression cassette. The Env proteins were seen in both cell lysates and supernatants. The levels of production
5 were significantly higher in cell supernatants for cells transfected with the synthetic Env expression cassettes of the present invention as compared to wild type.

Fractionation of the supernatants from mammalian cells transfected with the synthetic Env expression
10 cassettes showed that it provides superior production of Env proteins, relative to the wild-type Env sequences (Examples 2 and 3).

Efficient expression of these Env-containing polypeptides in mammalian cell lines provides the
15 following benefits: the Env polypeptides are free of baculovirus or other viral contaminants; production by established methods approved by the FDA; increased purity; greater yields (relative to native coding sequences); and a novel method of producing the Env-
20 containing polypeptides in CHO cells which is less feasible in the absence of the increased expression obtained using the constructs of the present invention.

Exemplary cell lines (e.g., mammalian, yeast, insect, etc.) include those described above in Section
25 2.1.3 for Gag-containing constructs. Further, appropriate vectors and control elements (e.g., promoters, enhancers, polyadenylation sequences, etc.) for any given cell type can be selected, as described above in Section 2.1.3, by one having ordinary skill in the art in view of the
30 teachings of the present specification and information known in the art about expression vectors. In addition, the recombinantly expressed polypeptides from synthetic Env-encoding expression cassettes are typically isolated and purified from lysed cells or culture media, as

described above for Gag-encoding expression cassettes. An exemplary purification is described in Example 4 and shown in Figure 60.

5 2.1.7 MODIFICATION OF HIV-1 TAT NUCLEIC ACID CODING
SEQUENCES

Another aspect of the present invention is the generation of HIV-1 tat protein coding sequences, and related sequences, having improved expression relative to
10 the corresponding wild-type sequence. Exemplary embodiments of the present invention are illustrated herein modifying the tat wild-type nucleotide sequence (SEQ ID NO:85, Figure 76) obtained from SF162 as described above. Exemplary synthetic tat constructs are
15 shown in SEQ ID NO:87, which depicts a tat construct encoding a full-length tat polypeptide from strain SF162; SEQ ID NO:88, which depicts a tat construct encoding a tat polypeptide having the cystein residue at position 22 changed; and SEQ ID NO:89, which depicts a tat construct
20 encoding the amino terminal portion of a tat polypeptide from strain SF162. The amino portion of the tat protein appears to contain many of the epitopes that induce an immune response. In addition, further modifications include replacement or deletion of the cystein residue at
25 position 22, for example with a valine residue, an alanine residue or a glycine residue (SEQ ID Nos: 88 and 89, Figures 79 and 81), see, e.g., Caputo et al. (1996) *Gene Ther.* 3:235. In Figure 81, which depicts a tat construct encoding the amino terminal portion of a tat
30 polypeptide, the nucleotides (nucleotides 64-66) encoding the cystein residues are underlined. The design and construction of suitable construct can be readily done using

the teachings of the present specification. As with Gag, pol, prot and Env, tat polypeptide coding sequences can be obtained from a variety of isolates (families, subtypes, etc.).

5 Modification of the tat polypeptide coding sequences result in improved expression relative to the wild-type coding sequences in a number of cell lines (e.g., mammalian, yeast, bacterial and insect cells). Tat polypeptide encoding sequences derived from these
10 variants can be optimized and tested for improved expression in mammals by following the teachings of the present specification (see the Examples, in particular Example 2).

15 Various forms of the different embodiments of the invention, described herein, may be combined. For example, polynucleotides may be derived from the polynucleotide sequences of the present invention, including, but not limited to, coding sequences for Gag polypeptides, Env polypeptides, polymerase polypeptides,
20 protease polypeptides, tat polypeptides, and reverse transcriptase polypeptides. Further, the polynucleotide coding sequences of the present invention may be combined into multi-cistronic expression cassettes where typically each coding sequence for each polypeptide is preceded by
25 IRES sequences.

2.2 PRODUCTION OF VIRUS-LIKE PARTICLES AND USE OF THE CONSTRUCTS OF THE PRESENT INVENTION TO CREATE PACKAGING CELL LINES

30 The group-specific antigens (Gag) of human immunodeficiency virus type-1 (HIV-1) self-assemble into noninfectious virus-like particles (VLP) that are released from various eucaryotic cells by budding (reviewed by Freed, E.O., Virology 251:1-15, 1998). The

synthetic expression cassettes of the present invention provide efficient means for the production of HIV-Gag virus-like particles (VLPs) using a variety of different cell types, including, but not limited to, mammalian
5 cells.

Viral particles can be used as a matrix for the proper presentation of an antigen entrapped or associated therewith to the immune system of the host. For example, U.S. Patent No. 4,722,840 describes hybrid particles
10 comprised of a particle-forming fragment of a structural protein from a virus, such as a particle-forming fragment of hepatitis B virus (HBV) surface antigen (HBsAg), fused to a heterologous polypeptide. Tindle et al., *Virology* (1994) 200:547-557, describes the production and use of
15 chimeric HBV core antigen particles containing epitopes of human papillomavirus (HPV) type 16 E7 transforming protein.

Adams et al., *Nature* (1987) 329:68-70, describes the recombinant production of hybrid HIVgpl20:Ty VLPs in
20 yeast and Brown et al., *Virology* (1994) 198:477-488, the production of chimeric proteins consisting of the VP2 protein of human parvovirus B19 and epitopes from human herpes simplex virus type 1, as well as mouse hepatitis virus A59. Wagner et al., (*Virology* (1994) 200:162-175,
25 Brand et al., *J. Virol. Meth.* (1995) 51:153-168; *Virology* (1996) 220:128-140) and Wolf, et al., (EP 0 449 116 A1, published 2 October 1991; WO 96/30523, published 3
October 1996) describe the assembly of chimeric HIV-1 p55Gag particles. U.S. Patent No. 5,503,833 describes
30 the use of rotavirus VP6 spheres for encapsulating and delivering therapeutic agents.

2.2.1 VLP PRODUCTION USING THE SYNTHETIC EXPRESSION
CASSETTES OF THE PRESENT INVENTION

Experiments performed in support of the present invention have demonstrated that the synthetic expression
5 cassettes of the present invention provide superior production of both protein and VLPs, relative to native coding sequences (Examples 7 and 15). Further, electron microscopic evaluation of VLP production (Examples 6 and 15, Figures 3A-B and 65A-F) showed that free and budding
10 immature virus particles of the expected size were produced by cells containing the synthetic expression cassettes.

Using the synthetic expression cassettes of the present invention, rather than native coding sequences,
15 for the production of virus-like particles provide several advantages. First, VLPs can be produced in enhanced quantity making isolation and purification of the VLPs easier. Second, VLPs can be produced in a variety of cell types using the synthetic expression
20 cassettes, in particular, mammalian cell-lines can be used for VLP production, for example, CHO cells. Production using CHO cells provides (i) VLP formation; (ii) correct myristylation and budding; (iii) absence of non-mammalian cell contaminants (e.g., insect viruses
25 and/or cells); and (iv) ease of purification. The synthetic expression cassettes of the present invention are also useful for enhanced expression in cell-types other than mammalian cell lines. For example, infection of insect cells with baculovirus vectors encoding the
30 synthetic expression cassettes resulted in higher levels of total protein yield and higher levels of VLP production (relative to wild-type coding sequences). Further, the final product from insect cells infected with the baculovirus-Gag synthetic expression cassettes

consistently contained lower amounts of contaminating insect proteins than the final product when wild-type coding sequences were used (Examples).

VLPs can spontaneously form when the particle-forming polypeptide of interest is recombinantly expressed in an appropriate host cell. Thus, the VLPs produced using the synthetic expression cassettes of the present invention are conveniently prepared using recombinant techniques. As discussed below, the Gag polypeptide encoding synthetic expression cassettes of the present invention can include other polypeptide coding sequences of interest (for example, Env, tat, rev, HIV protease, HIV polymerase, HCV core; see, Example 1). Expression of such synthetic expression cassettes yields VLPs comprising the product of the synthetic expression cassette, as well as, the polypeptide of interest.

Once coding sequences for the desired particle-forming polypeptides have been isolated or synthesized, they can be cloned into any suitable vector or replicon for expression. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. See, generally, Ausubel et al, *supra* or Sambrook et al, *supra*. The vector is then used to transform an appropriate host cell. Suitable recombinant expression systems include, but are not limited to, bacterial, mammalian, baculovirus/insect, vaccinia, Semliki Forest virus (SFV), Alphaviruses (such as, Sindbis, Venezuelan Equine Encephalitis (VEE)), mammalian, yeast and Xenopus expression systems, well known in the art. Particularly preferred expression systems are mammalian cell lines, vaccinia, Sindbis, insect and yeast systems.

For example, a number of mammalian cell lines are known in the art and include immortalized cell lines

available from the American Type Culture Collection (A.T.C.C.), such as, but not limited to, Chinese hamster ovary (CHO) cells, 293 cells, HeLa cells, baby hamster kidney (BHK) cells, mouse myeloma (SB20), monkey kidney cells (COS), as well as others. Similarly, bacterial hosts such as *E. coli*, *Bacillus subtilis*, and *Streptococcus spp.*, will find use with the present expression constructs. Yeast hosts useful in the present invention include *inter alia*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia guilliermondii*, *Pichia pastoris*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*. Insect cells for use with baculovirus expression vectors include, *inter alia*, *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni*. See, e.g., Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987). Fungal hosts include, for example, *Aspergillus*.

Viral vectors can be used for the production of particles in eucaryotic cells, such as those derived from the pox family of viruses, including vaccinia virus and avian poxvirus. Additionally, a vaccinia based infection/transfection system, as described in Tomei et al., *J. Virol.* (1993) 67:4017-4026 and Selby et al., *J. Gen. Virol.* (1993) 74:1103-1113, will also find use with the present invention. In this system, cells are first infected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the DNA of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus

recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translational machinery. Alternately, T7 can be added as a purified protein or enzyme as in the "Progenitor" system (Studier and Moffatt, *J. Mol. Biol.* (1986) 189:113-130). The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation product(s).

Depending on the expression system and host selected, the VLPs are produced by growing host cells transformed by an expression vector under conditions whereby the particle-forming polypeptide is expressed and VLPs can be formed. The selection of the appropriate growth conditions is within the skill of the art. If the VLPs are formed intracellularly, the cells are then disrupted, using chemical, physical or mechanical means, which lyse the cells yet keep the VLPs substantially intact. Such methods are known to those of skill in the art and are described in, e.g., *Protein Purification Applications: A Practical Approach*, (E.L.V. Harris and S. Angal, Eds., 1990).

The particles are then isolated (or substantially purified) using methods that preserve the integrity thereof, such as, by density gradient centrifugation, e.g., sucrose gradients, PEG-precipitation, pelleting, and the like (see, e.g., Kirnbauer et al. *J. Virol.* (1993) 67:6929-6936), as well as standard purification techniques including, e.g., ion exchange and gel filtration chromatography.

VLPs produced by cells containing the synthetic expression cassettes of the present invention can be used to elicit an immune response when administered to a subject. One advantage of the present invention is that VLPs can be produced by mammalian cells carrying the

synthetic expression cassettes at levels previously not possible. As discussed above, the VLPs can comprise a variety of antigens in addition to the Gag polypeptides (e.g., Env, tat, Gag-protease, Gag-polymerase, Gag-HCV-core). Purified VLPs, produced using the synthetic expression cassettes of the present invention, can be administered to a vertebrate subject, usually in the form of vaccine compositions. Combination vaccines may also be used, where such vaccines contain, for example, other subunit proteins derived from HIV or other organisms (e.g., env) or gene delivery vaccines encoding such antigens. Administration can take place using the VLPs formulated alone or formulated with other antigens. Further, the VLPs can be administered prior to, concurrent with, or subsequent to, delivery of the synthetic expression cassettes for DNA immunization (see below) and/or delivery of other vaccines. Also, the site of VLP administration may be the same or different as other vaccine compositions that are being administered. Gene delivery can be accomplished by a number of methods including, but are not limited to, immunization with DNA, alphavirus vectors, pox virus vectors, and vaccinia virus vectors.

VLP immune-stimulating (or vaccine) compositions can include various excipients, adjuvants, carriers, auxiliary substances, modulating agents, and the like. The immune stimulating compositions will include an amount of the VLP/antigen sufficient to mount an immunological response. An appropriate effective amount can be determined by one of skill in the art. Such an amount will fall in a relatively broad range that can be determined through routine trials and will generally be an amount on the order of about 0.1 μ g to about 1000 μ g,

more preferably about 1 μ g to about 300 μ g, of VLP/antigen.

A carrier is optionally present which is a molecule that does not itself induce the production of antibodies harmful to the individual receiving the composition.

Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycollic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus

particles. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; McGee JP, et al., *J Microencapsul.* 14(2):197-210, 1997; O'Hagan DT, et al., *Vaccine* 11(2):149-54, 1993. Such carriers are well known to those of ordinary skill in the art.

Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen may be conjugated to a bacterial toxoid, such as toxoid from diphtheria, tetanus, cholera, etc., as well as toxins derived from *E. coli*.

Such adjuvants include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc.; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (International Publication No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated

into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA),
(b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below)
5 either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and
(c) Ribⁱ™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group
10 consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (3) saponin adjuvants, such as Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particle generated therefrom such as
15 ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (IL-1, IL-2, etc.), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), beta chemokines (MIP, 1-
20 alpha, 1-beta Rantes, etc.); (6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63 (where lysine is substituted for the wild-type amino acid at position 63)
25 LT-R72 (where arginine is substituted for the wild-type amino acid at position 72), CT-S109 (where serine is substituted for the wild-type amino acid at position 109), and PT-K9/G129 (where lysine is substituted for the wild-type amino acid at position 9 and glycine
30 substituted at position 129) (see, e.g., International Publication Nos. W093/13202 and W092/19265); and (7)

other substances that act as immunostimulating agents to enhance the effectiveness of the composition.

Muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acteyl-normuramyl-L-alanyl-D-isogluatme (nor-MDP), N-acetylmuramyl-L-alanyl-D-isogluatminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

Dosage treatment with the VLP composition may be a single dose schedule or a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals, chosen to maintain and/or reinforce the immune response, for example at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the potency of the modality, the vaccine delivery employed, the need of the subject and be dependent on the judgment of the practitioner.

If prevention of disease is desired (e.g., reduction of symptoms, recurrences or of disease progression), the antigen carrying VLPs are generally administered prior to primary infection with the pathogen of interest. If treatment is desired, e.g., the reduction of symptoms or recurrences, the VLP compositions are generally administered subsequent to primary infection.

2.2.2 USING THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT INVENTION TO CREATE PACKAGING CELL LINES

A number of viral based systems have been developed for use as gene transfer vectors for mammalian host cells. For example, retroviruses (in particular,

lentiviral vectors) provide a convenient platform for gene delivery systems. A coding sequence of interest (for example, a sequence useful for gene therapy applications) can be inserted into a gene delivery vector and packaged in retroviral particles using techniques known in the art. Recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems have been described, including, for example, the following: (U.S. Patent No. 5,219,740; Miller et al. (1989) *Biotechniques* 7:980; Miller, A.D. (1990) *Human Gene Therapy* 1:5; Scarpa et al. (1991) *Virology* 180:849; Burns et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:8033; Boris-Lawrie et al. (1993) *Cur. Opin. Genet. Develop.* 3:102; GB 2200651; EP 0415731; EP 0345242; WO 89/02468; WO 89/05349; WO 89/09271; WO 90/02806; WO 90/07936; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; in U.S. 5,219,740; U.S. 4,405,712; U.S. 4,861,719; U.S. 4,980,289 and U.S. 4,777,127; in U.S. Serial No. 07/800,921; and in Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53:83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci USA* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Sequences useful for gene therapy applications include, but are not limited to, the following. Factor VIII cDNA, including derivatives and deletions thereof (International Publication Nos. WO 96/21035, WO 97/03193, WO 97/03194, WO 97/03195, and WO 97/03191). Factor IX cDNA (Kurachi et al. (1982) *Proc. Natl. Acad. Sci. USA* 79:6461-6464). Factor V cDNA can be obtained from pMT2-V (Jenny (1987) *Proc. Natl. Acad. Sci. USA* 84:4846, A.T.C.C. Deposit No. 40515). A full-length factor V

cDNA, or a B domain deletion or B domain substitution thereof, can be used. B domain deletions of factor V, include those reported by Marquette (1995) *Blood* 86:3026 and Kane (1990) *Biochemistry* 29:6762. Antithrombin III cDNA (Prochownik (1983) *J. Biol. Chem.* 258:8389, A.T.C.C. Deposit No. 57224/57225). Protein C encoding cDNA (Foster (1984) *Proc. Natl. Acad. Sci. USA* 81:4766; Beckmann (1985) *Nucleic Acids Res.* 13:5233). Prothrombin cDNA can be obtained by restriction enzyme digestion of a published vector (Degen (1983) *Biochemistry* 22:2087). The endothelial cell surface protein, thrombomodulin, is a necessary cofactor for the normal activation of protein C by thrombin. A soluble recombinant form has been described (Parkinson (1990) *J. Biol. Chem.* 265:12602; Jackman (1987) *Proc. Natl. Acad. Sci. USA* 84:6425; Shirai (1988) *J. Biochem.* 103:281; Wen (1987) *Biochemistry* 26:4350; Suzuki (1987) *EMBO J.* 6:1891, A.T.C.C. Deposit No. 61348, 61349).

Many genetic diseases caused by inheritance of defective genes result in the failure to produce normal gene products, for example, thalassemia, phenylketonuria, Lesch-Nyhan syndrome, severe combined immunodeficiency (SCID), hemophilia A and B, cystic fibrosis, Duchenne's Muscular Dystrophy, inherited emphysema and familial hypercholesterolemia (Mulligan et al. (1993) *Science* 260:926; Anderson et al. (1992) *Science* 256:808; Friedman et al. (1989) *Science* 244:1275). Although genetic diseases may result in the absence of a gene product, endocrine disorders, such as diabetes and hypopituitarism, are caused by the inability of the gene to produce adequate levels of the appropriate hormone insulin and human growth hormone respectively.

In one aspect, gene therapy employing the constructs and methods of the present invention involves the

introduction of normal recombinant genes into T cells so that new or missing proteins are produced by the T cells after introduction or reintroduction thereof into a patient. A number of genetic diseases have been selected
5 for treatment with gene therapy, including adenine deaminase deficiency, cystic fibrosis, α_1 -antitrypsin deficiency, Gaucher's syndrome, as well as non-genetic diseases.

In particular, Gaucher's syndrome is a genetic
10 disorder characterized by a deficiency of the enzyme glucocerebrosidase. This enzyme deficiency leads to the accumulation of glucocerebroside in the lysosomes of all cells in the body. For a review see *Science* 256:794 (1992) and Scriver et al., *The Metabolic Basis of*
15 *Inherited Disease*, 6th ed., vol. 2, page 1677). Thus, gene transfer vectors that express glucocerebrosidase can be constructed for use in the treatment of this disorder. Likewise, gene transfer vectors encoding lactase can be used in the treatment of hereditary lactose intolerance,
20 those expressing AD can be used for treatment of ADA deficiency, and gene transfer vectors encoding α_1 -antitrypsin can be used to treat α_1 -antitrypsin deficiency. See Ledley, F.D. (1987) *J. Pediatrics* 110:157-174, Verma, I. (Nov. 1987) *Scientific American*
25 pp. 68-84, and International Publication No. WO 95/27512 entitled "Gene Therapy Treatment for a Variety of Diseases and Disorders," for a description of gene therapy treatment of genetic diseases.

In still further embodiments of the invention,
30 nucleotide sequences which can be incorporated into a gene transfer vector include, but are not limited to, proteins associated with enzyme-deficiency disorders, such as the cystic fibrosis transmembrane regulator (see, for example, U.S. Patent No. 5,240,846 and Larrick et al.

(1991) *Gene Therapy Applications of Molecular Biology*, Elsevier, New York and adenosine deaminase (ADA) (see U.S. Patent No. 5,399,346); growth factors, or an agonist or antagonist of a growth factor (Bandara et al. (1992) *DNA and Cell Biology*, 11:227); one or more tumor suppressor genes such as p53, Rb, or C-CAMI (Kleiner et al. (1995) *Cancer Research* 55:2831); a molecule that modulates the immune system of an organism, such as a HLA molecule (Nabel et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:11307); a ribozyme (Larsson et al. (1996) *Virology* 219:161); a peptide nucleic acid (Hirshman et al. (1996) *J. Invest. Med.* 44:347); an antisense molecule (Bordier et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:9383) which can be used to down-regulate the expression or synthesis of aberrant or foreign proteins, such as HIV proteins or a wide variety of oncogenes such as p53 (Hesketh, *The Oncogene Facts Book*, Academic Press, New York, (1995); a biopharmaceutical agent or antisense molecule used to treat HIV-infection, such as an inhibitor of p24 (Nakashima et al. (1994) *Nucleic Acids Res.* 22:5004); or reverse-transcriptase (see, Bordier, *supra*).

Other proteins of therapeutic interest can be expressed *in vivo* by gene transfer vectors using the methods of the invention. For instance sustained *in vivo* expression of tissue factor inhibitory protein (TFPI) is useful for treatment of conditions including sepsis and DIC and in preventing reperfusion injury. (See International Publications Nos. WO 93/24143, WO 93/25230 and WO 96/06637). Nucleic acid sequences encoding various forms of TFPI can be obtained, for example, as described in US Patent Nos. 4,966,852; 5,106,833; and 5,466,783, and incorporated into the gene transfer vectors described herein.

Erythropoietin (EPO) and leptin can also be expressed in vivo from genetically modified T cells according to the methods of the invention. For instance EPO is useful in gene therapy treatment of a variety of disorders including anemia (see International Publication No. WO 95/13376 entitled "Gene Therapy for Treatment of Anemia"). Sustained delivery of leptin by the methods of the invention is useful in treatment of obesity. See International Publication No. WO 96/05309 for a description of the leptin gene and the use thereof in the treatment of obesity.

A variety of other disorders can also be treated by the methods of the invention. For example, sustained in vivo systemic production of apolipoprotein E or apolipoprotein A from genetically modified T cells can be used for treatment of hyperlipidemia (see Breslow et al. (1994) *Biotechnology* 12:365). Sustained production of angiotensin receptor inhibitor (Goodfriend et al. (1996) *N. Engl. J. Med.* 334:1469) can be provided by the methods described herein. As yet an additional example, the long term in vivo systemic production of angiostatin is useful in the treatment of a variety of tumors. (See O'Reilly et al. (1996) *Nature Med.* 2:689).

In other embodiments, gene transfer vectors can be constructed to encode a cytokine or other immunomodulatory molecule. For example, nucleic acid sequences encoding native IL-2 and gamma-interferon can be obtained as described in US Patent Nos. 4,738,927 and 5,326,859, respectively, while useful muteins of these proteins can be obtained as described in U.S. Patent No. 4,853,332. Nucleic acid sequences encoding the short and long forms of mCSF can be obtained as described in US Patent Nos. 4,847,201 and 4,879,227, respectively. In particular aspects of the invention, retroviral vectors

expressing cytokine or immunomodulatory genes can be produced as described herein (for example, employing the packaging cell lines of the present invention) and in International Application No. PCT US 94/02951, entitled

5 "Compositions and Methods for Cancer Immunotherapy."

Examples of suitable immunomodulatory molecules for use herein include the following: IL-1 and IL-2 (Karupiah et al. (1990) *J. Immunology* 144:290-298, Weber et al. (1987) *J. Exp. Med.* 166:1716-1733, Gansbacher et al. (1990) *J. Exp. Med.* 172:1217-1224, and U.S. Patent No. 4,738,927); IL-3 and IL-4 (Tepper et al. (1989) *Cell* 57:503-512, Golumbek et al. (1991) *Science* 254:713-716, and U.S. Patent No. 5,017,691); IL-5 and IL-6 (Brakenhof et al. (1987) *J. Immunol.* 139:4116-4121, and

15 International Publication No. WO 90/06370); IL-7 (U.S. Patent No. 4,965,195); IL-8, IL-9, IL-10, IL-11, IL-12, and IL-13 (*Cytokine Bulletin*, Summer 1994); IL-14 and IL-15; alpha interferon (Finter et al. (1991) *Drugs* 42:749-765, U.S. Patent Nos. 4,892,743 and 4,966,843,

20 International Publication No. WO 85/02862, Nagata et al. (1980) *Nature* 284:316-320, Familletti et al. (1981) *Methods in Enz.* 78:387-394, Twu et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:2046-2050, and Faktor et al. (1990) *Oncogene* 5:867-872); beta-interferon (Seif et al. (1991) *J. Virol.* 65:664-671); gamma-interferons (Radford et al. (1991) *The American Society of Hepatology* 20082015, Watanabe et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:9456-9460, Gansbacher et al. (1990) *Cancer Research* 50:7820-7825, Maio et al. (1989) *Can. Immunol.*

25 *Immunother.* 30:34-42, and U.S. Patent Nos. 4,762,791 and 4,727,138); G-CSF (U.S. Patent Nos. 4,999,291 and 4,810,643); GM-CSF (International Publication No. WO 85/04188); tumor necrosis factors (TNFs) (Jayaraman et al. (1990) *J. Immunology* 144:942-951); CD3 (Krissanen et

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al. (1987) *Immunogenetics* 26:258-266); ICAM-1 (Altman et al. (1989) *Nature* 338:512-514, Simmons et al. (1988) *Nature* 331:624-627); ICAM-2, LFA-1, LFA-3 (Wallner et al. (1987) *J. Exp. Med.* 166:923-932); MHC class I molecules, 5 MHC class II molecules, B7.1-.3, β_2 -microglobulin (Parnes et al. (1981) *Proc. Natl. Acad. Sci. USA* 78:2253-2257); chaperones such as calnexin; and MHC-linked transporter proteins or analogs thereof (Powis et al. (1991) *Nature* 354:528-531). Immunomodulatory factors may also be 10 agonists, antagonists, or ligands for these molecules. For example, soluble forms of receptors can often behave as antagonists for these types of factors, as can mutated forms of the factors themselves.

Nucleic acid molecules that encode the above- 15 described substances, as well as other nucleic acid molecules that are advantageous for use within the present invention, may be readily obtained from a variety of sources, including, for example, depositories such as the American Type Culture Collection, or from commercial 20 sources such as British Bio-Technology Limited (Cowley, Oxford England). Representative examples include BBG 12 (containing the GM-CSF gene coding for the mature protein of 127 amino acids), BBG 6 (which contains sequences encoding gamma interferon), A.T.C.C. Deposit No. 39656 25 (which contains sequences encoding TNF), A.T.C.C. Deposit No. 20663 (which contains sequences encoding alpha-interferon), A.T.C.C. Deposit Nos. 31902, 31902 and 39517 (which contain sequences encoding beta-interferon), A.T.C.C. Deposit No. 67024 (which contains a sequence 30 which encodes Interleukin-1b), A.T.C.C. Deposit Nos. 39405, 39452, 39516, 39626 and 39673 (which contain sequences encoding Interleukin-2), A.T.C.C. Deposit Nos. 59399, 59398, and 67326 (which contain sequences encoding Interleukin-3), A.T.C.C. Deposit No. 57592 (which

contains sequences encoding Interleukin-4), A.T.C.C. Deposit Nos. 59394 and 59395 (which contain sequences encoding Interleukin-5), and A.T.C.C. Deposit No. 67153 (which contains sequences encoding Interleukin-6).

5 Plasmids containing cytokine genes or immunomodulatory genes (International Publication Nos. WO 94/02951 and WO 96/21015) can be digested with appropriate restriction enzymes, and DNA fragments containing the particular gene of interest can be inserted into a gene transfer vector using standard molecular biology techniques. (See, e.g., Sambrook et al., *supra.*, or Ausubel et al. (eds) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley-Interscience).

10 Exemplary hormones, growth factors and other proteins which are useful for long term expression are described, for example, in European Publication No. 0437478B1, entitled "Cyclodextrin-Peptide Complexes." Nucleic acid sequences encoding a variety of hormones can be used, including those encoding human growth hormone, insulin, calcitonin, prolactin, follicle stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (HCG), and thyroid stimulating hormone (TSH). A variety of different forms of IGF-1 and IGF-2 growth factor polypeptides are also well known the art and can be incorporated into gene transfer vectors for long term expression *in vivo*. See, e.g., European Patent No. 0123228B1, published for grant September 19, 1993, entitled "Hybrid DNA Synthesis of Mature Insulin-like Growth Factors." As an additional example, the long term *in vivo* expression of different forms of fibroblast growth factor can also be effected employing the compositions and methods of invention. See, e.g., U.S. Patent Nos. 5,464,774, 5,155,214, and 4,994,559 for a description of different fibroblast growth factors.

Polynucleotide sequences coding for the above-described molecules can be obtained using recombinant methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene
5 from a vector known to include the same. For example, plasmids which contain sequences that encode altered cellular products may be obtained from a depository such as the A.T.C.C., or from commercial sources. Plasmids containing the nucleotide sequences of interest can be
10 digested with appropriate restriction enzymes, and DNA fragments containing the nucleotide sequences can be inserted into a gene transfer vector using standard molecular biology techniques.

Alternatively, cDNA sequences for use with the
15 present invention may be obtained from cells which express or contain the sequences, using standard techniques, such as phenol extraction and PCR of cDNA or genomic DNA. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain and isolate DNA.
20 Briefly, mRNA from a cell which expresses the gene of interest can be reverse transcribed with reverse transcriptase using oligo-dT or random primers. The single stranded cDNA may then be amplified by PCR (see U.S. Patent Nos. 4,683,202, 4,683,195 and 4,800,159, see
25 also *PCR Technology: Principles and Applications for DNA Amplification*, Erlich (ed.), Stockton Press, 1989)) using oligonucleotide primers complementary to sequences on either side of desired sequences.

The nucleotide sequence of interest can also be
30 produced synthetically, rather than cloned, using a DNA synthesizer (e.g., an Applied Biosystems Model 392 DNA Synthesizer, available from ABI, Foster City, California). The nucleotide sequence can be designed with the appropriate codons for the expression product

desired. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge (1981) *Nature* 292:756; Nambair et al. (1984) *Science* 223:1299; Jay et al. (1984) *J. Biol. Chem.* 259:6311.

The synthetic expression cassettes of the present invention can be employed in the construction of packaging cell lines for use with retroviral vectors.

One type of retrovirus, the murine leukemia virus, or "MLV", has been widely utilized for gene therapy applications (see generally Mann et al. (*Cell* 33:153, 1993), Cane and Mulligan (*Proc. Nat'l. Acad. Sci. USA* 81:6349, 1984), and Miller et al., *Human Gene Therapy* 1:5-14, 1990).

Lentiviral vectors typically, comprise a 5' lentiviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to one or more genes of interest, an origin of second strand DNA synthesis and a 3' lentiviral LTR, wherein the lentiviral vector contains a nuclear transport element. The nuclear transport element may be located either upstream (5') or downstream (3') of a coding sequence of interest. Within certain embodiments, the nuclear transport element is not RRE. Within one embodiment the packaging signal is an extended packaging signal. Within other embodiments the promoter is a tissue specific promoter, or, alternatively, a promoter such as CMV. Within other embodiments, the lentiviral vector further comprises an internal ribosome entry site.

A wide variety of lentiviruses may be utilized within the context of the present invention, including for example, lentiviruses selected from the group consisting of HIV, HIV-1, HIV-2, FIV and SIV.

In one embodiment of the present invention synthetic Env and/or Gag-polymerase expression cassettes are provided comprising a promoter and a sequence encoding synthetic Gag-polymerase (SEQ ID NO:6) and at least one
5 of vpr, vpu, nef or vif, wherein the promoter is operably linked to Gag-polymerase and vpr, vpu, nef or vif.

Within yet another aspect of the invention, host cells (e.g., packaging cell lines) are provided which contain any of the expression cassettes described herein.
10 For example, within one aspect packaging cell line are provided comprising an expression cassette that comprises a sequence encoding synthetic Env and/or Gag-polymerase, and a nuclear transport element, wherein the promoter is operably linked to the sequence encoding Env and/or Gag-
15 polymerase. Packaging cell lines may further comprise a promoter and a sequence encoding tat, rev, or an envelope, wherein the promoter is operably linked to the sequence encoding tat, rev, or, the envelope. The packaging cell line may further comprise a sequence
20 encoding any one or more of nef, vif, vpu or vpr.

In one embodiment, the expression cassette (carrying, for example, the synthetic Env, synthetic tat and/or synthetic Gag-polymerase) is stably integrated. The packaging cell line, upon introduction of a
25 lentiviral vector, typically produces viral particles. The promoter regulating expression of the synthetic expression cassette may be inducible. Typically, the packaging cell line, upon introduction of a lentiviral vector, produces viral particles that are essentially
30 free of replication competent virus.

Packaging cell lines are provided comprising an expression cassette which directs the expression of a synthetic Env (or Gag-polymerase) gene, an expression cassette which directs the expression of a Gag (or Env)

gene optimized for expression (e.g., Andre, S., et al.,
Journal of Virology 72(2):1497-1503, 1998; Haas, J., et
al., *Current Biology* 6(3):315-324, 1996). A lentiviral
vector is introduced into the packaging cell line to
5 produce a vector particle producing cell line.

As noted above, lentiviral vectors can be designed
to carry or express a selected gene(s) or sequences of
interest. Lentiviral vectors may be readily constructed
from a wide variety of lentiviruses (see RNA Tumor
10 Viruses, Second Edition, Cold Spring Harbor Laboratory,
1985). Representative examples of lentiviruses included
HIV, HIV-1, HIV-2, FIV and SIV. Such lentiviruses may
either be obtained from patient isolates, or, more
preferably, from depositories or collections such as the
15 American Type Culture Collection, or isolated from known
sources using available techniques.

Portions of the lentiviral gene delivery vectors (or
vehicles) may be derived from different viruses. For
example, in a given recombinant lentiviral vector, LTRs
20 may be derived from an HIV, a packaging signal from SIV,
and an origin of second strand synthesis from HrV-2.
Lentiviral vector constructs may comprise a 5' lentiviral
LTR, a tRNA binding site, a packaging signal, one or more
heterologous sequences, an origin of second strand DNA
25 synthesis and a 3' LTR, wherein said lentiviral vector
contains a nuclear transport element that is not RRE.

Briefly, Long Terminal Repeats ("LTRs") are
subdivided into three elements, designated U5, R and U3.
These elements contain a variety of signals which are
30 responsible for the biological activity of a retrovirus,
including for example, promoter and enhancer elements
which are located within U3. LTRs may be readily
identified in the provirus (integrated DNA form) due to
their precise duplication at either end of the genome.

As utilized herein, a 5' LTR should be understood to include a 5' promoter element and sufficient LTR sequence to allow reverse transcription and integration of the DNA form of the vector. The 3' LTR should be understood to include a polyadenylation signal, and sufficient LTR sequence to allow reverse transcription and integration of the DNA form of the vector.

The tRNA binding site and origin of second strand DNA synthesis are also important for a retrovirus to be biologically active, and may be readily identified by one of skill in the art. For example, retroviral tRNA binds to a tRNA binding site by Watson-Crick base pairing, and is carried with the retrovirus genome into a viral particle. The tRNA is then utilized as a primer for DNA synthesis by reverse transcriptase. The tRNA binding site may be readily identified based upon its location just downstream from the 5'LTR. Similarly, the origin of second strand DNA synthesis is, as its name implies, important for the second strand DNA synthesis of a retrovirus. This region, which is also referred to as the poly-purine tract, is located just upstream of the 3'LTR.

In addition to a 5' and 3' LTR, tRNA binding site, and origin of second strand DNA synthesis, recombinant retroviral vector constructs may also comprise a packaging signal, as well as one or more genes or coding sequences of interest. In addition, the lentiviral vectors have a nuclear transport element which, in preferred embodiments is not RRE. Representative examples of suitable nuclear transport elements include the element in Rous sarcoma virus (Ogert, et al., *J ViroL* 70, 3834-3843, 1996), the element in Rous sarcoma virus (Liu & Mertz, *Genes & Dev.*, 9, 1766-1789, 1995) and the element in the genome of simian retrovirus type I

(Zolotukhin, et al., *J Virol.* 68, 7944-7952, 1994). Other potential elements include the elements in the histone gene (Kedes, *Annu. Rev. Biochem.* 48, 837-870, 1970), the α -interferon gene (Nagata et al., *Nature* 287, 401-408, 1980), the β -adrenergic receptor gene (Koilkka, et al., *Nature* 329, 75-79, 1987), and the c-Jun gene (Hattorie, et al., *Proc. Natl. Acad. Sci. USA* 85, 9148-9152, 1988).

Recombinant lentiviral vector constructs typically lack both *Gag*-polymerase and *env* coding sequences. Recombinant lentiviral vector typically contain less than 20, preferably 15, more preferably 10, and most preferably 8 consecutive nucleotides found in *Gag*-polymerase or *env* genes. One advantage of the present invention is that the synthetic *Gag*-polymerase expression cassettes, which can be used to construct packaging cell lines for the recombinant retroviral vector constructs, have little homology to wild-type *Gag*-polymerase sequences and thus considerably reduce or eliminate the possibility of homologous recombination between the synthetic and wild-type sequences.

Lentiviral vectors may also include tissue-specific promoters to drive expression of one or more genes or sequences of interest. For example, lentiviral vector particles of the invention can contain a liver specific promoter to maximize the potential for liver specific expression of the exogenous DNA sequence contained in the vectors. Preferred liver specific promoters include the hepatitis B X-gene promoter and the hepatitis B core protein promoter. These liver specific promoters are preferably employed with their respective enhancers. The enhancer element can be linked at either the 5' or the 3' end of the nucleic acid encoding the sequences of interest. The hepatitis B X gene promoter and its

enhancer can be obtained from the viral genome as a 332 base pair *EcoRV*-*NcoI* DNA fragment employing the methods described in Twu, et al., *J Virol.* 61:3448-3453, 1987.

The hepatitis B core protein promoter can be obtained

5 from the viral genome as a 584 base pair *BamHI*-*BglIII* DNA fragment employing the methods described in Gerlach, et al., *Virol* 189:59-66, 1992. It may be necessary to

remove the negative regulatory sequence in the *BamHI*-

BglIII fragment prior to inserting it. Other liver

10 specific promoters include the AFP (alpha fetal protein)

gene promoter and the albumin gene promoter, as disclosed

in EP Patent Publication 0 415 731, the -1 antitrypsin

gene promoter, as disclosed in Rettenger, et al., *Proc.*

Natl. Acad. Sci. 91:1460-1464, 1994, the fibrinogen

15 gene promoter, the APO-A1 (Apolipoprotein A1) gene

promoter, and the promoter genes for liver transference

enzymes such as, for example, SGOT, SGPT and glutamyle

transferase. See also PCT Patent Publications WO

90/07936 and WO 91/02805 for a description of the use of

20 liver specific promoters in lentiviral vector particles.

Lentiviral vector constructs may be generated such

that more than one gene of interest is expressed. This

may be accomplished through the use of di- or oligo-

cistronic cassettes (e.g., where the coding regions are

25 separated by 80 nucleotides or less, see generally Levin

et al., *Gene* 108:167-174, 1991), or through the use of

Internal Ribosome Entry Sites ("IRES").

Packaging cell lines suitable for use with the above

described recombinant retroviral vector constructs may be

30 readily prepared given the disclosure provided herein.

Briefly, the parent cell line from which the packaging

cell line is derived can be selected from a variety of

mammalian cell lines, including for example, 293, RD, COS-7, CHO, BHK, VERO, HT1080, and myeloma cells.

After selection of a suitable host cell for the generation of a packaging cell line, one or more
5 expression cassettes are introduced into the cell line in order to complement or supply in trans components of the vector which have been deleted.

Representative examples of suitable expression cassettes have been described herein and include
10 synthetic Env, tat, Gag, synthetic Gag-protease, synthetic Gag-reverse transcriptase and synthetic Gag-polymerase expression cassettes, which comprise a promoter and a sequence encoding, e.g., Env, tat, or Gag-polymerase and at least one of vpr, vpu, nef or vif,
15 wherein the promoter is operably linked to Env, tat or Gag-polymerase and vpr, vpu, nef or vif. As described above, optimized Env, Gag and/or tat coding sequences may also be utilized in various combinations in the generation of packaging cell lines.

20 Utilizing the above-described expression cassettes, a wide variety of packaging cell lines can be generated. For example, within one aspect packaging cell line are provided comprising an expression cassette that comprises a sequence encoding synthetic HIV (e.g., Gag, Env, tat, Gag-polymerase, Gag-reverse transcriptase or Gag-
25 protease) polypeptide, and a nuclear transport element, wherein the promoter is operably linked to the sequence encoding the HIV polypeptide. Within other aspects, packaging cell lines are provided comprising a promoter and a sequence encoding Gag, tat, rev, or an envelope
30 (e.g., HIV env), wherein the promoter is operably linked to the sequence encoding Gag, tat, rev, or, the envelope. Within further embodiments, the packaging cell line may comprise a sequence encoding any one or more of nef, vif,

vpu or vpr. For example, the packaging cell line may contain only nef, vif, vpu, or vpr alone, nef and vif, nef and vpu, nef and vpr, vif and vpu, vif and vpr, vpu and vpr, nef vif and vpu, nef vif and vpr, nef vpu and vpr, vif vpu and vpr, or, all four of nef vif vpu and vpr.

In one embodiment, the expression cassette is stably integrated. Within another embodiment, the packaging cell line, upon introduction of a lentiviral vector, produces particles. Within further embodiments the promoter is inducible. Within certain preferred embodiments of the invention, the packaging cell line, upon introduction of a lentiviral vector, produces particles that are free of replication competent virus.

The synthetic cassettes containing optimized coding sequences are transfected into a selected cell line. Transfected cells are selected that (i) carry, typically, integrated, stable copies of the Gag, Pol, and Env coding sequences, and (ii) are expressing acceptable levels of these polypeptides (expression can be evaluated by methods known in the prior art, e.g., see Examples 1-4). The ability of the cell line to produce VLPs may also be verified (Examples 6, 7 and 15).

A sequence of interest is constructed into a suitable viral vector as discussed above. This defective virus is then transfected into the packaging cell line. The packaging cell line provides the viral functions necessary for producing virus-like particles into which the defective viral genome, containing the sequence of interest, are packaged. These VLPs are then isolated and can be used, for example, in gene delivery or gene therapy.

Further, such packaging cell lines can also be used to produce VLPs alone, which can, for example, be used as

adjuvants for administration with other antigens or in vaccine compositions. Also, co-expression of a selected sequence of interest encoding a polypeptide (for example, an antigen) in the packaging cell line can also result in the entrapment and/or association of the selected polypeptide in/with the VLPs.

2.3 DNA IMMUNIZATION AND GENE DELIVERY

A variety of polypeptide antigens can be used in the practice of the present invention. Polypeptide antigens can be included in DNA immunization constructs containing, for example, any of the synthetic expression cassettes described herein fused in-frame to a coding sequence for the polypeptide antigen, where expression of the construct results in VLPs presenting the antigen of interest. Antigens can be derived from a wide variety of viruses, bacteria, fungi, plants, protozoans and other parasites. For example, the present invention will find use for stimulating an immune response against a wide variety of proteins from the herpesvirus family, including proteins derived from herpes simplex virus (HSV) types 1 and 2, such as HSV-1 and HSV-2 gB, gD, gH, VP16 and VP22; antigens derived from varicella zoster virus (VZV), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) including CMV gB and gH; and antigens derived from other human herpesviruses such as HHV6 and HHV7. (See, e.g. Chee et al., *Cytomegaloviruses* (J.K. McDougall, ed., Springer-Verlag 1990) pp. 125-169, for a review of the protein coding content of cytomegalovirus; McGeoch et al., *J. Gen. Virol.* (1988) 69:1531-1574, for a discussion of the various HSV-1 encoded proteins; U.S. Patent No. 5,171,568 for a discussion of HSV-1 and HSV-2 gB and gD proteins and the genes encoding therefore; Baer et al., *Nature* (1984) 310:207-211, for the identification of

protein coding sequences in an EBV genome; and Davison and Scott, *J. Gen. Virol.* (1986) 67:1759-1816, for a review of VZV.)

5 Additionally, immune responses to antigens from the hepatitis family of viruses, including hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), the delta hepatitis virus (HDV), hepatitis E virus (HEV), and hepatitis G virus, can also be stimulated using the constructs of the present invention. By way of example,
10 the HCV genome encodes several viral proteins, including E1 (also known as E) and E2 (also known as E2/NSI), which will find use with the present invention (see, Houghton et al. *Hepatology* (1991) 14:381-388, for a discussion of HCV proteins, including E1 and E2). The δ -antigen from
15 HDV can also be used (see, e.g., U.S. Patent No. 5,389,528, for a description of the δ -antigen).

Similarly, influenza virus is another example of a virus for which the present invention will be particularly useful. Specifically, the envelope
20 glycoproteins HA and NA of influenza A are of particular interest for generating an immune response. Numerous HA subtypes of influenza A have been identified (Kawaoka et al., *Virology* (1990) 179:759-767; Webster et al. "Antigenic variation among type A influenza viruses," p.
25 127-168. In: P. Palese and D.W. Kingsbury (ed.), *Genetics of influenza viruses*. Springer-Verlag, New York).

Other antigens of particular interest to be used in the practice of the present invention include antigens and polypeptides derived therefrom from human
30 papillomavirus (HPV), such as one or more of the various early proteins including E6 and E7; tick-borne encephalitis viruses; and HIV-1 (also known as HTLV-III, LAV, ARV, etc.), including, but not limited to, antigens such as gp120, gp41, gp160, Gag and pol from a variety of

isolates including, but not limited to, HIV_{IIIB}, HIV_{SF2}, HIV-1_{SF162}, HIV-1_{SF170}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}, HIV-1_{CM235}, HIV-1_{US4}, other HIV-1 strains from diverse subtypes (e.g., subtypes, A through G, and O), HIV-2 strains and diverse subtypes (e.g., HIV-2_{UC1} and HIV-2_{UC2}). See, e.g., Myers, et al., Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico; Myers, et al., *Human Retroviruses and Aids*, 1990, Los Alamos, New Mexico: Los Alamos National Laboratory.

Proteins derived from other viruses will also find use in the claimed methods, such as without limitation, proteins from members of the families Picornaviridae (e.g., polioviruses, etc.); Caliciviridae; Togaviridae (e.g., rubella virus, dengue virus, etc.); Flaviviridae; Coronaviridae; Reoviridae; Birnaviridae; Rhabdoviridae (e.g., rabies virus, etc.); Filoviridae; Paramyxoviridae (e.g., mumps virus, measles virus, respiratory syncytial virus, etc.); Orthomyxoviridae (e.g., influenza virus types A, B and C, etc.); Bunyaviridae; Arenaviridae; Retroviridae, e.g., HTLV-I; HTLV-II; HIV-1; HIV-2; simian immunodeficiency virus (SIV) among others. See, e.g. Virology, 3rd Edition (W.K. Joklik ed. 1988); *Fundamental Virology*, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991; Virology, 3rd Edition (Fields, BN, DM Knipe, PM Howley, Editors, 1996, Lippincott-Raven, Philadelphia, PA) for a description of these and other viruses.

Particularly preferred bacterial antigens are derived from organisms that cause diphtheria, tetanus, pertussis, meningitis, and other pathogenic states, including, without limitation, antigens derived from *Corynebacterium diphtheriae*, *Clostridium tetani*, *Bordetella pertussis*, *Neisseria meningitidis*, including serotypes *Meningococcus* A, B, C, Y and WI35 (MenA, B, C, Y and WI35), *Haemophilus influenza* type B (Hib), and

Helicobacter pylori. Examples of parasitic antigens include those derived from organisms causing malaria, tuberculosis, and Lyme disease.

Furthermore, the methods described herein provide
5 means for treating a variety of malignant cancers. For example, the system of the present invention can be used to enhance both humoral and cell-mediated immune responses to particular proteins specific to a cancer in question, such as an activated oncogene, a fetal antigen,
10 or an activation marker. Such tumor antigens include any of the various MAGEs (melanoma associated antigen E), including MAGE 1, 2, 3, 4, etc. (Boon, T. *Scientific American* (March 1993):82-89); any of the various tyrosinases; MART 1 (melanoma antigen recognized by T
15 cells), mutant ras; mutant p53; p97 melanoma antigen; CEA (carcinoembryonic antigen), among others.

DNA immunization using synthetic expression cassettes of the present invention has been demonstrated to be efficacious (Examples 8 and 10-12). Animals were
20 immunized with both the synthetic expression cassette and the wild type expression cassette. The results of the immunizations with plasmid-DNAs showed that the synthetic expression cassettes provide a clear improvement of immunogenicity relative to the native expression
25 cassettes. Also, the second boost immunization induced a secondary immune response, for example after two to eight weeks. Further, the results of CTL assays showed increased potency of synthetic expression cassettes for induction of cytotoxic T-lymphocyte (CTL) responses by
30 DNA immunization.

It is readily apparent that the subject invention can be used to mount an immune response to a wide variety of antigens and hence to treat or prevent a large number of diseases.

2.3.1 DELIVERY OF THE SYNTHETIC EXPRESSION CASSETTES OF THE
PRESENT INVENTION

Polynucleotide sequences coding for the above-described molecules can be obtained using recombinant
5 methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene from a vector known to include the same. The sequences can be analyzed by conventional sequencing techniques. Furthermore, the desired gene can be isolated directly
10 from cells and tissues containing the same, using standard techniques, such as phenol extraction and PCR of cDNA or genomic DNA. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain, isolate and sequence DNA. Once the sequence is known, the gene
15 of interest can also be produced synthetically, rather than cloned. The nucleotide sequence can be designed with the appropriate codons for the particular amino acid sequence desired. In general, one will select preferred codons for the intended host in which the sequence will
20 be expressed. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature* (1981) 292:756; Nambair et al., *Science* (1984) 223:1299; Jay et al., *J. Biol. Chem.* (1984) 259:6311; Stemmer, W.P.C., (1995) *Gene*
25 164:49-53.

Next, the gene sequence encoding the desired antigen can be inserted into a vector containing a synthetic expression cassette of the present invention (e.g., see
30 Example 1 for construction of various exemplary synthetic expression cassette). The antigen is inserted into the synthetic coding sequence such that when the combined sequence is expressed it results in the production of VLPs comprising the polypeptide and/or the antigen of

interest. Insertions can be made within the Gag coding sequence or at either end of the coding sequence (5', amino terminus of the expressed polypeptide; or 3', carboxy terminus of the expressed polypeptide -- e.g., see Example 1) (Wagner, R., et al., *Arch Virol.* 127:117-137, 1992; Wagner, R., et al., *Virology* 200:162-175, 1994; Wu, X., et al., *J. Virol.* 69(6):3389-3398, 1995; Wang, C-T., et al., *Virology* 200:524-534, 1994; Chazal, N., et al., *Virology* 68(1):111-122, 1994; Griffiths, J.C., et al., *J. Virol.* 67(6):3191-3198, 1993; Reicin, A.S., et al., *J. Virol.* 69(2):642-650, 1995).

Up to 50% of the coding sequences of p55Gag can be deleted without affecting the assembly to virus-like particles and expression efficiency (Borsetti, A., et al., *J. Virol.* 72(11):9313-9317, 1998; Gamier, L., et al., *J Virol* 72(6):4667-4677, 1998; Zhang, Y., et al., *J Virol* 72(3):1782-1789, 1998; Wang, C., et al., *J Virol* 72(10):7950-7959, 1998). In one embodiment of the present invention, immunogenicity of the high level expressing synthetic p55GagMod and p55GagProtMod expression cassettes can be increased by the insertion of different structural or non-structural HIV antigens, multiepitope cassettes, or cytokine sequences into deleted, mutated or truncated regions of p55GagMod sequence. In another embodiment of the present invention, immunogenicity of the high level expressing synthetic Env expression cassettes can be increased by the insertion of different structural or non-structural HIV antigens, multiepitope cassettes, or cytokine sequences into deleted regions of gp120Mod, gp140Mod or gp160Mod sequences. Such deletions may be generated following the teachings of the present invention and information available to one of ordinary skill in the art. One possible advantage of this approach, relative to using full-length modified Env

sequences fused to heterologous polypeptides, can be higher expression/secretion efficiency and/or higher immunogenicity of the expression product. Such deletions may be generated following the teachings of the present invention and information available to one of ordinary skill in the art. One possible advantage of this approach, relative to using full-length Env, Gag or Tat sequences fused to heterologous polypeptides, can be higher expression/secretion efficiency and/or immunogenicity of the expression product.

When sequences are added to the amino terminal end of Gag (for example, when using the synthetic p55GagMod expression cassette of the present invention), the polynucleotide can contain coding sequences at the 5' end that encode a signal for addition of a myristic moiety to the Gag-containing polypeptide (e.g., sequences that encode Met-Gly).

The ability of Gag-containing polypeptide constructs to form VLPs can be empirically determined following the teachings of the present specification.

HIV polypeptide/antigen synthetic expression cassettes include control elements operably linked to the coding sequence, which allow for the expression of the gene *in vivo* in the subject species. For example, typical promoters for mammalian cell expression include the SV40 early promoter, a CMV promoter such as the CMV immediate early promoter, the mouse mammary tumor virus LTR promoter, the adenovirus major late promoter (Ad MLP), and the herpes simplex virus promoter, among others. Other nonviral promoters, such as a promoter derived from the murine metallothionein gene, will also find use for mammalian expression. Typically, transcription termination and polyadenylation sequences will also be present, located 3' to the translation stop

codon. Preferably, a sequence for optimization of initiation of translation, located 5' to the coding sequence, is also present. Examples of transcription terminator/polyadenylation signals include those derived
5 from SV40, as described in Sambrook et al., *supra*, as well as a bovine growth hormone terminator sequence.

Enhancer elements may also be used herein to increase expression levels of the mammalian constructs. Examples include the SV40 early gene enhancer, as
10 described in Dijkema et al., *EMBO J.* (1985) 4:761, the enhancer/promoter derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus, as described in Gorman et al., *Proc. Natl. Acad. Sci. USA* (1982b) 79:6777 and
15 elements derived from human CMV, as described in Boshart et al., *Cell* (1985) 41:521, such as elements included in the CMV intron A sequence.

Furthermore, plasmids can be constructed which include a chimeric antigen-coding gene sequences, encoding, e.g., multiple antigens/epitopes of interest,
20 for example derived from a single or from more than one viral isolate.

Typically the antigen coding sequences precede or follow the synthetic coding sequences and the chimeric transcription unit will have a single open reading frame
25 encoding both the antigen of interest and the synthetic Gag coding sequences. Alternatively, multi-cistronic cassettes (e.g., bi-cistronic cassettes) can be constructed allowing expression of multiple antigens from a single mRNA using the EMCV IRES, or the like. Lastly,
30 antigens can be encoded on separate transcripts from independent promoters on a single plasmid or other vector.

Once complete, the constructs are used for nucleic acid immunization or the like using standard gene

delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Patent Nos. 5,399,346, 5,580,859, 5,589,466. Genes can be delivered either directly to the vertebrate subject or, alternatively, delivered ex vivo, to cells derived from the subject and the cells reimplanted in the subject.

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. Selected sequences can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems have been described (U.S. Patent No. 5,219,740; Miller and Rosman, *BioTechniques* (1989) 7:980-990; Miller, A.D., *Human Gene Therapy* (1990) 1:5-14; Scarpa et al., *Virology* (1991) 180:849-852; Burns et al., *Proc. Natl. Acad. Sci. USA* (1993) 90:8033-8037; and Boris-Lawrie and Temin, *Cur. Opin. Genet. Develop.* (1993) 3:102-109.

A number of adenovirus vectors have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham, *J. Virol.* (1986) 57:267-274; Bett et al., *J. Virol.* (1993) 67:5911-5921; Mittereder et al., *Human Gene Therapy* (1994) 5:717-729; Seth et al., *J. Virol.* (1994) 68:933-940; Barr et al., *Gene Therapy* (1994) 1:51-58; Berkner, K.L. *BioTechniques* (1988) 6:616-629; and Rich et al., *Human Gene Therapy* (1993) 4:461-476).

Additionally, various adeno-associated virus (AAV) vector systems have been developed for gene delivery.

AAV vectors can be readily constructed using techniques well known in the art. See, e.g., U.S. Patent Nos.

5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 (published 23 January 1992) and WO 93/03769

- 5 (published 4 March 1993); Lebkowski et al., *Molec. Cell. Biol.* (1988) 8:3988-3996; Vincent et al., *Vaccines* 90 (1990) (Cold Spring Harbor Laboratory Press); Carter, B.J. *Current Opinion in Biotechnology* (1992) 3:533-539; Muzyczka, N. *Current Topics in Microbiol. and Immunol.* 10 (1992) 158:97-129; Kotin, R.M. *Human Gene Therapy* (1994) 5:793-801; Shelling and Smith, *Gene Therapy* (1994) 1:165-169; and Zhou et al., *J. Exp. Med.* (1994) 179:1867-1875.

- Another vector system useful for delivering the polynucleotides of the present invention is the enterically administered recombinant poxvirus vaccines described by Small, Jr., P.A., et al. (U.S. Patent No. 5,676,950, issued October 14, 1997).
- 15

- Additional viral vectors which will find use for delivering the nucleic acid molecules encoding the antigens of interest include those derived from the pox family of viruses, including vaccinia virus and avian poxvirus. By way of example, vaccinia virus recombinants expressing the genes can be constructed as follows. The DNA encoding the particular synthetic Gag/antigen coding sequence is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the coding sequences of interest into the viral genome. The resulting TK⁻recombinant can be selected by culturing the
- 20
- 25
- 30

cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver the genes. Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer protective immunity when administered to non-avian species. The use of an avipox vector is particularly desirable in human and other mammalian species since members of the avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia viruses. See, e.g., WO 91/12882; WO 89/03429; and WO 92/03545.

Molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael et al., *J. Biol. Chem.* (1993) 268:6866-6869 and Wagner et al., *Proc. Natl. Acad. Sci. USA* (1992) 89:6099-6103, can also be used for gene delivery.

Members of the Alphavirus genus, such as, but not limited to, vectors derived from the Sindbis, Semliki Forest, and Venezuelan Equine Encephalitis viruses, will also find use as viral vectors for delivering the polynucleotides of the present invention (for example, a synthetic Gag- or Env-polypeptide encoding expression cassette as described in Example 14 below). For a description of Sindbis-virus derived vectors useful for the practice of the instant methods, see, Dubensky et al., *J. Virol.* (1996) 70:508-519; and International Publication Nos. WO 95/07995 and WO 96/17072; as well as, Dubensky, Jr., T.W., et al., U.S. Patent No. 5,843,723,

issued December 1, 1998, and Dubensky, Jr., T.W., U.S. Patent No. 5,789,245, issued August 4, 1998.

A vaccinia based infection/transfection system can be conveniently used to provide for inducible, transient
5 expression of the coding sequences of interest (for example, a synthetic Gag/HCV-core expression cassette) in a host cell. In this system, cells are first infected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase
10 displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the polynucleotide of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus
15 recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation products. See,
20 e.g., Elroy-Stein and Moss, *Proc. Natl. Acad. Sci. USA* (1990) 87:6743-6747; Fuerst et al., *Proc. Natl. Acad. Sci. USA* (1986) 83:8122-8126.

As an alternative approach to infection with vaccinia or avipox virus recombinants, or to the delivery
25 of genes using other viral vectors, an amplification system can be used that will lead to high level expression following introduction into host cells. Specifically, a T7 RNA polymerase promoter preceding the coding region for T7 RNA polymerase can be engineered.
30 Translation of RNA derived from this template will generate T7 RNA polymerase which in turn will transcribe more template. Concomitantly, there will be a cDNA whose expression is under the control of the T7 promoter. Thus, some of the T7 RNA polymerase generated from

translation of the amplification template RNA will lead to transcription of the desired gene. Because some T7 RNA polymerase is required to initiate the amplification, T7 RNA polymerase can be introduced into cells along with the template(s) to prime the transcription reaction. The polymerase can be introduced as a protein or on a plasmid encoding the RNA polymerase. For a further discussion of T7 systems and their use for transforming cells, see, e.g., International Publication No. WO 94/26911; Studier and Moffatt, *J. Mol. Biol.* (1986) 189:113-130; Deng and Wolff, *Gene* (1994) 143:245-249; Gao et al., *Biochem. Biophys. Res. Commun.* (1994) 200:1201-1206; Gao and Huang, *Nuc. Acids Res.* (1993) 21:2867-2872; Chen et al., *Nuc. Acids Res.* (1994) 22:2114-2120; and U.S. Patent No. 5,135,855.

The synthetic expression cassette of interest can also be delivered without a viral vector. For example, the synthetic expression cassette can be packaged as DNA or RNA in liposomes prior to delivery to the subject or to cells derived therefrom. Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed DNA to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight, *Biochim. Biophys. Acta.* (1991) 1097:1-17; Straubinger et al., in *Methods of Enzymology* (1983), Vol. 101, pp. 512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations, with cationic liposomes particularly preferred. Cationic liposomes have been shown to mediate intracellular

delivery of plasmid DNA (Felgner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416); mRNA (Malone et al., *Proc. Natl. Acad. Sci. USA* (1989) 86:6077-6081); and purified transcription factors (Debs et al., *J. Biol. Chem.* (1990) 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416). Other commercially available lipids include (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; PCT Publication No. WO 90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as, from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See, e.g., Straubinger et al., in *METHODS OF*

IMMUNOLOGY (1983), Vol. 101, pp. 512-527; Szoka et al.,
Proc. Natl. Acad. Sci. USA (1978) 75:4194-4198;
Papahadjopoulos et al., Biochim. Biophys. Acta (1975)
394:483; Wilson et al., Cell (1979) 17:77; Deamer and
5 Bangham, Biochim. Biophys. Acta (1976) 443:629; Ostro et
al., Biochem. Biophys. Res. Commun. (1977) 76:836; Fraley
et al., Proc. Natl. Acad. Sci. USA (1979) 76:3348; Enoch
and Strittmatter, Proc. Natl. Acad. Sci. USA (1979)
76:145; Fraley et al., J. Biol. Chem. (1980) 255:10431;
10 Szoka and Papahadjopoulos, Proc. Natl. Acad. Sci. USA
(1978) 75:145; and Schaefer-Ridder et al., Science (1982)
215:166.

The DNA and/or protein antigen(s) can also be
delivered in cochleate lipid compositions similar to
15 those described by Papahadjopoulos et al., Biochem.
Biophys. Acta. (1975) 394:483-491. See, also, U.S.
Patent Nos. 4,663,161 and 4,871,488.

The synthetic expression cassette of interest (e.g.,
any of the synthetic expression cassettes described in
20 Example 1) may also be encapsulated, adsorbed to, or
associated with, particulate carriers. Such carriers
present multiple copies of a selected antigen to the
immune system and promote migration, trapping and
retention of antigens in local lymph nodes. The
25 particles can be taken up by professional antigen
presenting cells such as macrophages and dendritic cells,
and/or can enhance antigen presentation through other
mechanisms such as stimulation of cytokine release.
Examples of particulate carriers include those derived
30 from polymethyl methacrylate polymers, as well as
microparticles derived from poly(lactides) and
poly(lactide-co-glycolides), known as PLG. See, e.g.,
Jeffery et al., Pharm. Res. (1993) 10:362-368; McGee JP,

et al., *J Microencapsul.* 14(2):197-210, 1997; O'Hagan DT, et al., *Vaccine* 11(2):149-54, 1993.

Furthermore, other particulate systems and polymers can be used for the *in vivo* or *ex vivo* delivery of the gene of interest. For example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules, are useful for transferring a nucleic acid of interest. Similarly, DEAE dextran-mediated transfection, calcium phosphate precipitation or precipitation using other insoluble inorganic salts, such as strontium phosphate, aluminum silicates including bentonite and kaolin, chromic oxide, magnesium silicate, talc, and the like, will find use with the present methods. See, e.g., Felgner, P.L., *Advanced Drug Delivery Reviews* (1990) 5:163-187, for a review of delivery systems useful for gene transfer. Peptoids (Zuckerman, R.N., et al., U.S. Patent No. 5,831,005, issued November 3, 1998) may also be used for delivery of a construct of the present invention.

Additionally, biolistic delivery systems employing particulate carriers such as gold and tungsten, are especially useful for delivering synthetic expression cassettes of the present invention. The particles are coated with the synthetic expression cassette(s) to be delivered and accelerated to high velocity, generally under a reduced atmosphere, using a gun powder discharge from a "gene gun." For a description of such techniques, and apparatuses useful therefore, see, e.g., U.S. Patent Nos. 4,945,050; 5,036,006; 5,100,792; 5,179,022; 5,371,015; and 5,478,744. Also, needle-less injection systems can be used (Davis, H.L., et al, *Vaccine* 12:1503-1509, 1994; Bioject, Inc., Portland, OR).

Recombinant vectors carrying a synthetic expression cassette of the present invention are formulated into compositions for delivery to the vertebrate subject. These compositions may either be prophylactic (to prevent infection) or therapeutic (to treat disease after infection). The compositions will comprise a "therapeutically effective amount" of the gene of interest such that an amount of the antigen can be produced *in vivo* so that an immune response is generated in the individual to which it is administered. The exact amount necessary will vary depending on the subject being treated; the age and general condition of the subject to be treated; the capacity of the subject's immune system to synthesize antibodies; the degree of protection desired; the severity of the condition being treated; the particular antigen selected and its mode of administration, among other factors. An appropriate effective amount can be readily determined by one of skill in the art. Thus, a "therapeutically effective amount" will fall in a relatively broad range that can be determined through routine trials.

The compositions will generally include one or more "pharmaceutically acceptable excipients or vehicles" such as water, saline, glycerol, polyethyleneglycol, hyaluronic acid, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, surfactants and the like, may be present in such vehicles. Certain facilitators of immunogenicity or of nucleic acid uptake and/or expression can also be included in the compositions or coadministered, such as, but not limited to, bupivacaine, cardiotoxin and sucrose.

Once formulated, the compositions of the invention can be administered directly to the subject (e.g., as

described above) or, alternatively, delivered ex vivo, to cells derived from the subject, using methods such as those described above. For example, methods for the ex vivo delivery and reimplantation of transformed cells into a subject are known in the art and can include, e.g., dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, lipofectamine and LT-1 mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) (with or without the corresponding antigen) in liposomes, and direct microinjection of the DNA into nuclei.

Direct delivery of synthetic expression cassette compositions in vivo will generally be accomplished with or without viral vectors, as described above, by injection using either a conventional syringe, needleless devices such as Bioject® or a gene gun, such as the Accell® gene delivery system (PowderJect Technologies, Inc., Oxford, England). The constructs can be delivered (e.g., injected) either subcutaneously, epidermally, intradermally, intramuscularly, intravenous, intramucosally (such as nasally, rectally and vaginally), intraperitoneally or orally. Delivery of DNA into cells of the epidermis is particularly preferred as this mode of administration provides access to skin-associated lymphoid cells and provides for a transient presence of DNA in the recipient. Other modes of administration include oral ingestion and pulmonary administration, suppositories, needle-less injection, transcutaneous and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule.

2.3.2 EX VIVO DELIVERY OF THE SYNTHETIC EXPRESSION
CASSETTES OF THE PRESENT INVENTION

In one embodiment, T cells, and related cell types (including but not limited to antigen presenting cells, such as, macrophage, monocytes, lymphoid cells, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof), can be used for ex vivo delivery of the synthetic expression cassettes of the present invention. T cells can be isolated from peripheral blood lymphocytes (PBLs) by a variety of procedures known to those skilled in the art. For example, T cell populations can be "enriched" from a population of PBLs through the removal of accessory and B cells. In particular, T cell enrichment can be accomplished by the elimination of non-T cells using anti-MHC class II monoclonal antibodies. Similarly, other antibodies can be used to deplete specific populations of non-T cells. For example, anti-Ig antibody molecules can be used to deplete B cells and anti-MacI antibody molecules can be used to deplete macrophages.

T cells can be further fractionated into a number of different subpopulations by techniques known to those skilled in the art. Two major subpopulations can be isolated based on their differential expression of the cell surface markers CD4 and CD8. For example, following the enrichment of T cells as described above, CD4⁺ cells can be enriched using antibodies specific for CD4 (see Coligan et al., *supra*). The antibodies may be coupled to a solid support such as magnetic beads. Conversely, CD8⁺ cells can be enriched through the use of antibodies specific for CD4 (to remove CD4⁺ cells), or can be isolated by the use of CD8 antibodies coupled to a solid support. CD4

lymphocytes from HIV-1 infected patients can be expanded ex vivo, before or after transduction as described by Wilson et. al. (1995) *J. Infect. Dis.* 172:88.

5 Following purification of T cells, a variety of methods of genetic modification known to those skilled in the art can be performed using non-viral or viral-based gene transfer vectors constructed as described herein. For example, one such approach involves transduction of
10 the purified T cell population with vector-containing supernatant of cultures derived from vector producing cells. A second approach involves co-cultivation of an irradiated monolayer of vector-producing cells with the purified T cells. A third approach involves a similar
15 co-cultivation approach; however, the purified T cells are pre-stimulated with various cytokines and cultured 48 hours prior to the co-cultivation with the irradiated vector producing cells. Pre-stimulation prior to such transduction increases effective gene transfer (Nolta et
20 al. (1992) *Exp. Hematol.* 20:1065). Stimulation of these cultures to proliferate also provides increased cell populations for re-infusion into the patient. Subsequent to co-cultivation, T cells are collected from the vector producing cell monolayer, expanded, and frozen in liquid
25 nitrogen.

Gene transfer vectors, containing one or more synthetic expression cassette of the present invention (associated with appropriate control elements for delivery to the isolated T cells) can be assembled using
30 known methods.

Selectable markers can also be used in the construction of gene transfer vectors. For example, a marker can be used which imparts to a mammalian cell transduced with the gene transfer vector resistance to a

cytotoxic agent. The cytotoxic agent can be, but is not limited to, neomycin, aminoglycoside, tetracycline, chloramphenicol, sulfonamide, actinomycin, netropsin, distamycin A, anthracycline, or pyrazinamide. For example, neomycin phosphotransferase II imparts resistance to the neomycin analogue geneticin (G418).

The T cells can also be maintained in a medium containing at least one type of growth factor prior to being selected. A variety of growth factors are known in the art which sustain the growth of a particular cell type. Examples of such growth factors are cytokine mitogens such as rIL-2, IL-10, IL-12, and IL-15, which promote growth and activation of lymphocytes. Certain types of cells are stimulated by other growth factors such as hormones, including human chorionic gonadotropin (hCG) and human growth hormone. The selection of an appropriate growth factor for a particular cell population is readily accomplished by one of skill in the art.

For example, white blood cells such as differentiated progenitor and stem cells are stimulated by a variety of growth factors. More particularly, IL-3, IL-4, IL-5, IL-6, IL-9, GM-CSF, M-CSF, and G-CSF, produced by activated T_H and activated macrophages, stimulate myeloid stem cells, which then differentiate into pluripotent stem cells, granulocyte-monocyte progenitors, eosinophil progenitors, basophil progenitors, megakaryocytes, and erythroid progenitors. Differentiation is modulated by growth factors such as GM-CSF, IL-3, IL-6, IL-11, and EPO.

Pluripotent stem cells then differentiate into lymphoid stem cells, bone marrow stromal cells, T cell progenitors, B cell progenitors, thymocytes, T_H Cells, T_c cells, and B cells. This differentiation is modulated by

growth factors such as IL-3, IL-4, IL-6, IL-7, GM-CSF, M-CSF, G-CSF, IL-2, and IL-5.

Granulocyte-monocyte progenitors differentiate to monocytes, macrophages, and neutrophils. Such
5 differentiation is modulated by the growth factors GM-CSF, M-CSF, and IL-8. Eosinophil progenitors differentiate into eosinophils. This process is modulated by GM-CSF and IL-5.

The differentiation of basophil progenitors into
10 mast cells and basophils is modulated by GM-CSF, IL-4, and IL-9. Megakaryocytes produce platelets in response to GM-CSF, EPO, and IL-6. Erythroid progenitor cells differentiate into red blood cells in response to EPO.

Thus, during activation by the CD3-binding agent, T
15 cells can also be contacted with a mitogen, for example a cytokine such as IL-2. In particularly preferred embodiments, the IL-2 is added to the population of T cells at a concentration of about 50 to 100 $\mu\text{g/ml}$. Activation with the CD3-binding agent can be carried out
20 for 2 to 4 days.

Once suitably activated, the T cells are genetically modified by contacting the same with a suitable gene transfer vector under conditions that allow for transfection of the vectors into the T cells. Genetic
25 modification is carried out when the cell density of the T cell population is between about 0.1×10^6 and 5×10^6 , preferably between about 0.5×10^6 and 2×10^6 . A number of suitable viral and nonviral-based gene transfer vectors have been described for use herein.

30 After transduction, transduced cells are selected away from non-transduced cells using known techniques. For example, if the gene transfer vector used in the transduction includes a selectable marker which confers resistance to a cytotoxic agent, the cells can be

contacted with the appropriate cytotoxic agent, whereby non-transduced cells can be negatively selected away from the transduced cells. If the selectable marker is a cell surface marker, the cells can be contacted with a binding agent specific for the particular cell surface marker, whereby the transduced cells can be positively selected away from the population. The selection step can also entail fluorescence-activated cell sorting (FACS) techniques, such as where FACS is used to select cells from the population containing a particular surface marker, or the selection step can entail the use of magnetically responsive particles as retrievable supports for target cell capture and/or background removal.

More particularly, positive selection of the transduced cells can be performed using a FACS cell sorter (e.g. a FACSVantage™ Cell Sorter, Becton Dickinson Immunocytometry Systems, San Jose, CA) to sort and collect transduced cells expressing a selectable cell surface marker. Following transduction, the cells are stained with fluorescent-labeled antibody molecules directed against the particular cell surface marker. The amount of bound antibody on each cell can be measured by passing droplets containing the cells through the cell sorter. By imparting an electromagnetic charge to droplets containing the stained cells, the transduced cells can be separated from other cells. The positively selected cells are then harvested in sterile collection vessels. These cell sorting procedures are described in detail, for example, in the FACSVantage™ Training Manual, with particular reference to sections 3-11 to 3-28 and 10-1 to 10-17.

Positive selection of the transduced cells can also be performed using magnetic separation of cells based on expression of a particular cell surface marker. In such

separation techniques, cells to be positively selected are first contacted with specific binding agent (e.g., an antibody or reagent that interacts specifically with the cell surface marker). The cells are then contacted with
5 retrievable particles (e.g., magnetically responsive particles) which are coupled with a reagent that binds the specific binding agent (that has bound to the positive cells). The cell-binding agent-particle complex can then be physically separated from non-labeled cells,
10 for example using a magnetic field. When using magnetically responsive particles, the labeled cells can be retained in a container using a magnetic field while the negative cells are removed. These and similar separation procedures are known to those of ordinary
15 skill in the art.

Expression of the vector in the selected transduced cells can be assessed by a number of assays known to those skilled in the art. For example, Western blot or Northern analysis can be employed depending on the nature
20 of the inserted nucleotide sequence of interest. Once expression has been established and the transformed T cells have been tested for the presence of the selected synthetic expression cassette, they are ready for infusion into a patient via the peripheral blood stream.

25 The invention includes a kit for genetic modification of an ex vivo population of primary mammalian cells. The kit typically contains a gene transfer vector coding for at least one selectable marker and at least one synthetic expression cassette contained
30 in one or more containers, ancillary reagents or hardware, and instructions for use of the kit.

EXPERIMENTAL

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not
5 intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should,
10 of course, be allowed for.

Example 1Generation of Synthetic Gag and Env Expression Cassettes

15 A. Modification of HIV-1 Gag, Gag-protease, Gag-reverse transcriptase and Gag-polymerase Nucleic Acid Coding Sequences

The Gag (SEQ ID NO:1), Gag-protease (SEQ ID NO:2), Gag-polymerase (SEQ ID NO:3), and Gag-reverse
20 transcriptase (SEQ ID NO:77) coding sequences were selected from the HIV-1SF2 strain (Sanchez-Pescador, R., et al., *Science* 227(4686): 484-492, 1985; Luciw, P.A., et al. U.S. Patent No. 5,156,949, issued October 20, 1992; Luciw, P.A., et al., U.S. Patent No. 5,688,688, November
25 18, 1997). These sequences were manipulated to maximize expression of their gene products.

First, the HIV-1 codon usage pattern was modified so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human
30 genes. The HIV codon usage reflects a high content of the nucleotides A or T of the codon-triplet. The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a high AU content in the RNA and in a decreased translation ability and instability of the

mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Gag-encoding sequences were modified to be comparable to codon usage found in highly expressed human genes.

5 Figure 11 presents a comparison of the percent A-T content for the cDNAs of stable versus unstable RNAs (comparison window size = 50). Human IFN γ mRNA is known to (i) be unstable, (ii) have a short half-life, and (iii) have a high A-U content. Human GAPDH
10 (glyceraldehyde-3-phosphate dehydrogenase) mRNA is known to (i) be a stable RNA, and (i) have a low A-U content. In Figure 11, the percent A-T content of these two sequences are compared to the percent A-T content of native HIV-1SF2 Gag cDNA and to the synthetic Gag cDNA
15 sequence of the present invention. The top two panels of the figure show the percent A-T content over the length of the sequences for IFN γ and native Gag. The bottom two panels of the figure show the percent A-T content over the length of the sequences for GAPDH and the synthetic
20 Gag. Experiments performed in support of the present invention showed that the synthetic Gag sequences were capable of higher level of protein production (see the Examples) than the native Gag sequences. The data in Figure 11 suggest that one reason for this increased
25 production may be increased stability of the mRNA corresponding to the synthetic Gag coding sequences versus the mRNA corresponding to the native Gag coding sequences.

30 Second, there are inhibitory (or instability) elements (INS) located within the coding sequences of the Gag and Gag-protease coding sequences (Schneider R, et al., *J Virol.* 71(7):4892-4903, 1997). RRE is a secondary RNA structure that interacts with the HIV encoded Rev-protein to overcome the expression down-regulating

effects of the INS. To overcome the requirement for post-transcriptional activating mechanisms of RRE and Rev, and to enhance independent expression of the Gag polypeptide, the INS were inactivated by introducing multiple point mutations that did not alter the reading frame of the encoded proteins. Figure 1 shows the original SF2 Gag sequence, the location of the INS sequences, and the modifications made to the INS sequences to reduce their effects.

For the Gag-protease sequence (wild type, SEQ ID NO:2; synthetic, SEQ ID NOs:5, 78 and 79), the changes in codon usage were restricted to the regions up to the -1 frameshift and starting again at the end of the Gag reading frame (Figure 2; the region indicated in lower case letters in Figure 2 is the unmodified region). Further, inhibitory (or instability) elements (INS) located within the coding sequences of the Gag-protease polypeptide coding sequence were altered as well (indicated in Figure 2). The synthetic coding sequences were assembled by the Midland Certified Reagent Company (Midland, Texas).

Modification of the Gag-polymerase sequences (wild type, SEQ ID NO:3; synthetic, SEQ ID NO:6) and Gag-reverse transcriptase sequences (SEQ ID NOs:80 through 84) include similar modifications as described for Gag-protease in order to preserve the frameshift region. Locations of the inactivation sites and changes to the sequence to alter the inactivation sites are presented in Figure 12 for the native HIV-1_{SF2} Gag-polymerase sequence.

In one embodiment of the invention, the full length polymerase coding region of the Gag-polymerase sequence is included with the synthetic Gag sequences in order to increase the number of epitopes for virus-like particles expressed by the synthetic, optimized Gag expression

cassette. Because synthetic HIV-1 Gag-polymerase expresses the potentially deleterious functional enzymes reverse transcriptase (RT) and integrase (INT) (in addition to the structural proteins and protease), it is important to inactivate RT and INT functions. Several in-frame deletions in the RT and INT reading frame can be made to achieve catalytic nonfunctional enzymes with respect to their RT and INT activity. {Jay. A. Levy (Editor) (1995) *The Retroviridae*, Plenum Press, New York. ISBN 0-306-45033X. Pages 215-20; Grimison, B. and Laurence, J. (1995), *Journal Of Acquired Immune Deficiency Syndromes and Human Retrovirology* 9(1):58-68; Wakefield, J. K., et al., (1992) *Journal Of Virology* 66(11):6806-6812; Esnouf, R., et al., (1995) *Nature Structural Biology* 2(4):303-308; Maignan, S., et al., (1998) *Journal Of Molecular Biology* 282(2):359-368; Katz, R. A. and Skalka, A. M. (1994) *Annual Review Of Biochemistry* 73 (1994); Jacobo-Molina, A., et al., (1993) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 90(13):6320-6324; Hickman, A. B., et al., (1994) *Journal Of Biological Chemistry* 269(46):29279-29287; Goldgur, Y., et al., (1998) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 95(16):9150-9154; Goette, M., et al., (1998) *Journal Of Biological Chemistry* 273(17):10139-10146; Gorton, J. L., et al., (1998) *Journal of Virology* 72(6):5046-5055; Engelman, A., et al., (1997) *Journal Of Virology* 71(5):3507-3514; Dyda, F., et al., *Science* 266(5193):1981-1986; Davies, J. F., et al., (1991) *Science* 252(5002):88-95; Bujacz, G., et al., (1996) *Febs Letters* 398(2-3):175-178; Beard, W. A., et al., (1996) *Journal Of Biological Chemistry* 271(21):12213-12220; Kohlstaedt, L. A., et al., (1992)

Science 256(5065):1783-1790; Krug, M. S. and Berger, S. L. (1991) *Biochemistry* 30(44):10614-10623; Mazumder, A., et al., (1996) *Molecular Pharmacology* 49(4):621-628; Palaniappan, C., et al., (1997) *Journal Of Biological Chemistry* 272(17):11157-11164; Rodgers, D. W., et al., (1995) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 92(4):1222-1226; Sheng, N. and Dennis, D. (1993) *Biochemistry* 32(18):4938-4942; Spence, R. A., et al., (1995) *Science* 267(5200):988-993.}

Furthermore selected B- and/or T-cell epitopes can be added to the Gag-polymerase constructs within the deletions of the RT- and INT-coding sequence to replace and augment any epitopes deleted by the functional modifications of RT and INT. Alternately, selected B- and T-cell epitopes (including CTL epitopes) from RT and INT can be included in a minimal VLP formed by expression of the synthetic Gag or synthetic GagProt cassette, described above. (For descriptions of known HIV B- and T-cell epitopes see, HIV Molecular Immunology Database CTL Search Interface; Los Alamos Sequence Compendia, 1987-1997; Internet address: <http://hiv-web.lanl.gov/immunology/index.html>.)

The resulting modified coding sequences are presented as a synthetic Gag expression cassette (SEQ ID NO:4), a synthetic Gag-protease expression cassette (SEQ ID NOs:5, 78 and 79), and a synthetic Gag-polymerase expression cassette (SEQ ID NO:6). Synthetic expression cassettes containing codon modifications in the reverse transcriptase region are shown in SEQ ID NOs:80 through 84. An alignment of selected sequences is presented in Figure 7. A common region (Gag-common; SEQ ID NO:9) extends from position 1 to position 1262.

The synthetic DNA fragments for Gag and Gag-protease were cloned into the following expression vectors:

pCMVKm2, for transient expression assays and DNA immunization studies, the pCMVKm2 vector was derived from pCMV6a (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986) and comprises a kanamycin selectable marker, a
5 ColE1 origin of replication, a CMV promoter enhancer and Intron A, followed by an insertion site for the synthetic sequences described below followed by a polyadenylation signal derived from bovine growth hormone -- the pCMVKm2 vector differs from the pCMV-link vector only in that a
10 polylinker site was inserted into pCMVKm2 to generate pCMV-link (Figure 14, polylinker at positions 1646 to 1697); pESN2dhfr (Figure 13A) and pCMVPLEdhfr (also known as pCMVIII as shown in Figure 13B), for expression in Chinese Hamster Ovary (CHO) cells; and, pAcc13, a shuttle
15 vector for use in the Baculovirus expression system (pAcc13, was derived from pAcc12 which was described by Munemitsu S., et al., *Mol Cell Biol.* 10(11):5977-5982, 1990).

A restriction map for vector pCMV-link is presented
20 in Figure 14. In the figure, the CMV promoter (CMV IE ENH/PRO), bovine growth hormone terminator (BGH pA), kanamycin selectable marker (kan), and a ColE1 origin of replication (ColE1 ori) are indicated. A polycloning site is also indicated in the figure following the CMV
25 promoter sequences.

A restriction map for vector pESN2dhfr is presented in Figure 13A. In the figure, the CMV promoter (pCMV, hCMVIE), bovine growth hormone terminator (BGHpA), SV40 origin of replication (SV40ori), neomycin selectable
30 marker (Neo), SV40 polyA (SV40pA), Adenovirus 2 late promoter (Ad2VLP), and the murine dhfr gene (mu dhfr) are indicated. A polycloning site is also indicated in the figure following the CMV promoter sequences.

Briefly, construction of pCMVPLEdhfr (pCMVIII) was as follows. To construct a DHFR cassette, the EMCV IRES (internal ribosome entry site) leader was PCR-amplified from pCite-4a+ (Novagen, Inc., Milwaukee, WI) and
5 inserted into pET-23d (Novagen, Inc., Milwaukee, WI) as an Xba-Nco fragment to give pET-EMCV. The *dhfr* gene was PCR-amplified from pESN2dhfr to give a product with a Gly-Gly-Gly-Ser spacer in place of the translation stop codon and inserted as an Nco-BamH1 fragment to give pET-
10 E-DHFR. Next, the attenuated *neo* gene was PCR amplified from a pSV2Neo (Clontech, Palo Alto, CA) derivative and inserted into the unique BamH1 site of pET-E-DHFR to give pET-E-DHFR/Neo_(m2). Then, the bovine growth hormone terminator from pCDNA3 (Invitrogen, Inc., Carlsbad, CA)
15 was inserted downstream of the *neo* gene to give pET-E-DHFR/Neo_(m2)BGHT. The EMCV-*dhfr*/*neo* selectable marker cassette fragment was prepared by cleavage of pET-E-DHFR/Neo_(m2)BGHT. The CMV enhancer/promoter plus Intron A was transferred from pCMV6a (Chapman et al., *Nuc. Acids*
20 *Res.* (1991) 19:3979-3986) as a HindIII-SalI fragment into pUC19 (New England Biolabs, Inc., Beverly, MA). The vector backbone of pUC19 was deleted from the NdeI to the SapI sites. The above described DHFR cassette was added to the construct such that the EMCV IRES followed the CMV
25 promoter to produce the final construct. The vector also contained an *amp^r* gene and an SV40 origin of replication.

Selected pCMVKm2 vectors containing the synthetic expression cassettes have been designated as follows: pCMVKm2.GagMod.SF2, pCMVKm2.GagprotMod.SF2, and
30 pCMVKm2.GagpolMod.SF2, pCMVKm2.GagprotMod.SF2.GP1 (SEQ ID NO:78) and pCMVKm2.GagprotMod.SF2.GP2 (SEQ ID NO:79). Other exemplary Gag-encoding expressing cassettes are shown in the Figures and as Sequence Listings.

B. Modification of HIV-1 Gag/Hepatitis C Core Chimeric Protein Nucleic Acid Coding Sequences Generation of Synthetic Expression Cassettes

To facilitate the ligation of the Gag and HCV core coding sequences, PCR amplification was employed. The synthetic p55Gag expression cassette was used as a PCR template with the following primers: GAG5 (SEQ ID NO:11) and P55-SAL3 (SEQ ID NO:12). The PCR amplification was conducted at 55°C for 25 cycles using Stratagene's Pfu polymerase. The resulting PCR product was rendered free of nucleotides and primers using the Promega PCR clean-up kit and then subjected to EcoRI and SalI digestions. For HCV core coding sequences, the following primers were used with an HCV template (Houghton, M., et al., U.S. Patent No. 5,714,596, issued February 3, 1998; Houghton, M., et al., U.S. Patent No. 5,712,088, issued January 27, 1998; Houghton, M., et al., U.S. Patent No. 5,683,864, issued November 4, 1997; Weiner, A.J., et al., U.S. Patent No. 5,728,520, issued March 17, 1998; Weiner, A.J., et al., U.S. Patent No. 5,766,845, issued June 16, 1998; Weiner, A.J., et al., U.S. Patent No. 5,670,152, issued September 23, 1997): CORESAL 5 (SEQ ID NO:13) and 173CORE (SEQ ID NO:14) using the conditions outlined above. The purified product was digested with SalI and BamHI restriction enzymes. The digested Gag and HCV core PCR products were ligated into the pCMVKm2 vector digested with EcoRI and BamHI. Ligation of the PCR products at the SalI site resulted in a direct fusion of the final amino acid of p55Gag to the second amino acid of HCV core, serine. Amino acid 173 of core is a serine and is followed immediately by a TAG termination codon. The sequence of the fusion clone was confirmed. The pCMVKm2 vector containing the synthetic expression

cassette was designated as pCMVKm2.GagModHCVcore.

The EcoRI-BamHI fragment of p55Gag-core 173 was also cloned into EcoRI-BamHI-digested pAcC13 for baculovirus expression. Western blots confirmed expression and sucrose gradient sedimentation along with electron microscopy confirmed particle formation. To generate the above clone but containing the synthetic Gag sequences (instead of wild-type), the following steps were performed: pCMVKm2-modified p55Gag was used as template for PCR amplification with MS65 (SEQ ID NO:15) and MS66 (SEQ ID NO:16) primers. The region amplified corresponds to the BspHI and SalI sites at the C-terminus of synthetic Gag sequence. The amplification product was digested with BspHI and SalI and ligated to SalI/BamHI digested pCMV-link along with the Sal/BspHI fragment from pCMV-Km-p55modGag, representing the amino terminal end of modified Gag, and the SalI/BamHI fragment from pCMV-p55Gag-core173. Thereafter, a T4-blunted-SalI partial/BamHI fragment was ligated into pAcC4-SmaI/BamHI to generate pAcC4-p55GagMod-core173 (containing the synthetic sequence presented as SEQ ID NO:7).

C. Defining of the Major Homology Region (MHR) of HIV-1 p55Gag

The Major Homology Region (MHR) of HIV-1 p55 (Gag) is located in the p24-CA sequence of Gag. It is a conserved stretch of 20 amino acids (SEQ ID NO:19). The position in the wild type HIV-1_{sf2} Gag protein is from aa 286-305 and spans a region from nucleotides 856-915 in the native HIV-1_{sf2} Gag DNA-sequence. The position in the synthetic Gag protein is from aa 288-307 and spans a region from nucleotides 862-921 for the synthetic Gag DNA-sequence. The nucleotide sequence for the MHR in the synthetic

GagMod.SF2 is presented as SEQ ID NO:20. Mutations or deletions in the amino acid sequence of the MHR can severely impair particle production (Borsetti, A., et al., *J. Virol.* 72(11):9313-9317, 1998; Mammano, F., et al., *J Virol* 68(8):4927-4936, 1994).

Percent identity to the MHR nucleotide sequence can be determined, for example, using the MacDNAsis program (Hitachi Software Engineering America Limited, South San Francisco, CA), Higgins algorithm, with the following exemplary parameters: gap penalty = 5, no. of top diagonals = 5, fixed gap penalty = 5, K-tuple = 2, window size = 5, and floating gap penalty = 10.

D. Generation of Synthetic Env Expression Cassettes

Env coding sequences of the present invention include, but are not limited to, polynucleotide sequences encoding the following HIV-encoded polypeptides: gp160, gp140, and gp120 (see, e.g., U.S. Patent No. 5,792,459 for a description of the HIV-1_{SF2} ("SF2") Env polypeptide). The relationships between these polypeptides is shown schematically in Figure 15 (in the figure: the polypeptides are indicated as lines, the amino and carboxy termini are indicated on the gp160 line; the open circle represents the oligomerization domain; the open square represents a transmembrane spanning domain (TM); and "c" represents the location of a cleavage site, in gp140.mut the "X" indicates that the cleavage site has been mutated such that it no longer functions as a cleavage site). The polypeptide gp160 includes the coding sequences for gp120 and gp41. The polypeptide gp41 is comprised of several domains including an oligomerization domain (OD) and a transmembrane spanning domain (TM). In the native envelope, the oligomerization domain is required for the

non-covalent association of three gp41 polypeptides to form a trimeric structure: through non-covalent interactions with the gp41 trimer (and itself), the gp120 polypeptides are also organized in a trimeric structure.

5 A cleavage site (or cleavage sites) exists approximately between the polypeptide sequences for gp120 and the polypeptide sequences corresponding to gp41. This cleavage site(s) can be mutated to prevent cleavage at the site. The resulting gp140 polypeptide corresponds to

10 a truncated form of gp160 where the transmembrane spanning domain of gp41 has been deleted. This gp140 polypeptide can exist in both monomeric and oligomeric (i.e. trimeric) forms by virtue of the presence of the oligomerization domain in the gp41 moiety. In the

15 situation where the cleavage site has been mutated to prevent cleavage and the transmembrane portion of gp41 has been deleted the resulting polypeptide product is designated "mutated" gp140 (e.g., gp140.mut). As will be apparent to those in the field, the cleavage site can be

20 mutated in a variety of ways. The native amino acid sequence in the SF162 cleavage sites is: APTKAKRRVVQREKR (SEQ ID NO:21), where KAKRR (SEQ ID NO:22) is termed the "second" site and REKR (SEQ ID NO:23) is the "first site". Exemplary mutations include the following

25 constructs: gp140.mut7.modSF162 which encodes the amino acid sequence APTKAISSVVQSEKS (SEQ ID NO:24) in the cleavage site region; gp140.mut8.modSF162 which encodes the amino acid sequence APTIAISSVVQSEKS (SEQ ID NO:25) in the cleavage site region and gp140mut.modSF162 which
30 encodes the amino acid sequence APTKAKRRVVQREKS (SEQ ID NO:26). Mutations are denoted in bold. The native amino acid sequence in the US4 cleavage sites is:

APTQAKRRVVQREKR (SEQ ID NO:27), where QAKRR (SEQ ID NO:28) is termed the "second" site and REKR (SEQ ID

NO:23) is the "first site". Exemplary mutations include the following construct: gp140.mut.modUS4 which encodes the amino acid sequence APTQAKRRVVQREKS (SEQ ID NO:29) in the cleavage site region. Mutations are denoted in bold.

5

E. Modification of HIV-1 Env (Envelope) Nucleic Acid Coding Sequences

In one embodiment of the present invention, wild-type Env coding sequences were selected from the HIV-1_{SF162} ("SF162") strain (Cheng-Mayer (1989) *PNAS USA* 86:8575-8579). These SF162 sequences were as follows: gp120, SEQ ID NO:30 (Fig. 16); gp140, SEQ ID NO:31 (Fig. 17); and gp160, SEQ ID NO:32 (Fig. 18).

10 In another embodiment of the present invention, wild-type Env coding sequences were selected from the HIV-US4 strain (Mascola, et al. (1994) *J. Infect. Dis.* 169:48-54). These US4 sequences were as follows: gp120, SEQ ID NO:51 (Fig. 38); gp140, SEQ ID NO:52 (Fig. 39); and gp160, SEQ ID NO:53 (Fig. 40).

15 These Env coding sequences were manipulated to maximize expression of their gene products.

First, the wild-type coding region was modified in one or more of the following ways. In one embodiment, sequences encoding hypervariable regions of Env, particularly V1 and/or V2 were deleted. In other 25 embodiments, mutations were introduced into sequences encoding the cleavage site in Env to abrogate the enzymatic cleavage of oligomeric gp140 into gp120 monomers. (See, e.g., Earl et al. (1990) *PNAS USA* 87:648-652; Earl et al. (1991) *J. Virol.* 65:31-41). In 30 yet other embodiments, hypervariable region(s) were deleted, N-glycosylation sites were removed and/or cleavage sites mutated.

Second, the HIV-1 codon usage pattern was modified

so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes. The HIV codon usage reflects a high content of the nucleotides A or T in the codon-triplet. The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Env coding sequences were modified to be comparable to codon usage found in highly expressed human genes.

Figures 22A-22H present comparisons of the percent A-T content for the cDNAs of stable versus unstable RNAs (comparison window size = 50). Human IFN γ mRNA is known to (i) be unstable, (ii) have a short half-life, and (iii) have a high A-U content. Human GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA is known to (i) be a stable RNA, and (i) have a low A-U content. In Figures 22A-H, the percent A-T content of these two sequences are compared to the percent A-T content of (1) native HIV-1 US4 Env gp160 cDNA, a synthetic US4 Env gp160 cDNA sequence (i.e., having modified codons) of the present invention; and (2) native HIV-1 SF162 Env gp160 cDNA, a synthetic SF162 Env gp160 cDNA sequence (i.e., having modified codons) of the present invention. Figures 22A-H show the percent A-T content over the length of the sequences for IFN γ (Figures 22C and 22G); native gp160 Env US4 and SF162 (Figures 22A and 22E, respectively); GAPDH (Figures 22D and 22H); and the synthetic gp160 Env for US4 and SF162 (Figures 22B and 22F). Experiments performed in support of the present invention showed that the synthetic Env sequences were capable of higher level of protein production (see the Examples) than the native Env sequences. The data in Figures 22A-H suggest that one reason for this increased

production is increased stability of the mRNA corresponding to the synthetic Env coding sequences versus the mRNA corresponding to the native Env coding sequences.

5 To create the synthetic coding sequences of the present invention the gene cassettes were designed to comprise the entire coding sequence of interest. Synthetic gene cassettes were constructed by oligonucleotide synthesis and PCR amplification to
10 generate gene fragments. Primers were chosen to provide convenient restriction sites for subcloning. The resulting fragments were then ligated to create the entire desired sequence which was then cloned into an appropriate vector. The final synthetic sequences were
15 (i) screened by restriction endonuclease digestion and analysis, (ii) subjected to DNA sequencing in order to confirm that the desired sequence had been obtained and (iii) the identity and integrity of the expressed protein confirmed by SDS-PAGE and Western blotting (See,
20 Examples. The synthetic coding sequences were assembled at Chiron Corp. or by the Midland Certified Reagent Company (Midland, Texas).

 Exemplary modified coding sequences are presented as synthetic Env expression cassettes in Table 1A and 1B.
25 The following expression cassettes (i) have unique, terminal *EcoRI* and *XbaI* cloning sites; (ii) include Kozak sequences to promote optimal translation; (iii) tPA signal sequences (to direct the ENV polypeptide to the cell membrane, see, e.g., Chapman et al., *infra*); (iv)
30 open reading frames optimized for expression in mammalian cells; and (v) a translational stop signal codon.

Table 1A: Exemplary Synthetic Env Expression
Cassettes(SF162)

| | Expression Cassette | Seq Id | Further Information |
|----|----------------------------------|-----------|--|
| 5 | gp120 SF162 | 30 | wild-type; Figure 16 |
| | gp140 SF162 | 31 | wild-type; Figure 17 |
| | gp160 SF162 | 32 | wild-type; Figure 18 |
| | gp120.modSF162 | 33 | none; Figure 19 |
| | gp120.modSF162.delV2 | 34 | deleted V2 loop; Figure 20 |
| 10 | gp120.modSF162.delV1/V2 | 35 | deleted V1 and V2; Figure 21 |
| | gp140.modSF162 | 36 | none; Figure 23 |
| | gp140.modSF162.delV2 | 37 | deleted V2 loop; Figure 24 |
| | gp140.modSF162.delV1/V2 | 38 | deleted V1 and V2; Figure 25 |
| | gp140.mut.modSF162 | 39 | mutated cleavage site; Fig. 26 |
| 15 | gp140.mut.modSF162.delV2 | 40 | deleted V2; mutated cleavage site; Figure 27 |
| | gp140.mut.modSF162.delV1/V 2 | 41 | deleted V1 & V2; mutated cleavage site; Figure 28 |
| | gp140.mut7.modSF162 | 42 | mutated cleavage site; Fig. 29 |
| | gp140.mut7.modSF162.delV2 | 43 | mutated cleavage site; deleted V2; Figure 30 |
| 20 | gp140.mut7.modSF162.delV1/ V2 | 44 | mutated cleavage site; deleted V1 and V2; Figure 31 |
| | gp140.mut8.modSF162 | 45 | mutated cleavage site; Fig. 32 |
| | gp140.mut8.modSF162.delV2 | 46 | mutated cleavage site; deleted V2; Figure 33 |
| 25 | gp140.mut8.modSF162.delV1/ V2 | 47 | mutated cleavage site; deleted V1 and V2; Figure 34 |
| | gp160.modSF162 | 48 | none; Figure 35 |
| | gp160.modSF162.delV2 | 49 | deleted V2 loop; Figure 36 |
| | gp160.modSF162.delV1/V2 | 50 | deleted V1 & V2; Figure 37 |

Table 1B:

Exemplary Synthetic Env Expression Cassettes (US4)

| | Expression Cassette | Seq Id | Further Information |
|----|----------------------------------|-----------|--|
| 5 | gp120 US4 | 51 | wild-type; Figure 38 |
| | gp140 US4 | 52 | wild-type; Figure 39 |
| | gp160 US4 | 53 | wild-type; Figure 40 |
| | gp120.modUS4 | 54 | none; Figure 41 |
| | gp120.modUS4.del 128-194 | 55 | deletion in V1 and V2 regions; Figure 42 |
| | gp140.modUS4 | 56 | none; Figure 43 |
| 10 | gp140.mut.modUS4 | 57 | mutated cleavage site; Figure 44 |
| | gp140TM.modUS4 | 58 | native transmembrane region; Figure 45 |
| | gp140.modUS4.delV1/V2 | 59 | deleted V1 and V2; Figure 46 |
| | gp140.modUS4.delV2 | 60 | deleted V1; Figure 47 |
| | gp140.mut.modUS4.delV1/V2 | 61 | mutated cleavage site; deleted V1 and V2; Figure 48 |
| 15 | gp140.modUS4.del 128-194 | 62 | deletion in V1 and V2 regions; Figure 49 |
| | gp140.mut.modUS4.del 128- 194 | 63 | mutated cleavage site; deletion in V1 and V2 regions; Figure 50 |
| | gp160.modUS4 | 64 | none; Figure 51 |
| | gp160.modUS4.delV1 | 65 | deleted V1; Figure 52 |
| 20 | gp160.modUS4.delV2 | 66 | deleted V2; Figure 53 |
| | gp160.modUS4.delV1/V2 | 67 | deleted V1 and V2; Figure 54 |
| | gp160.modUS4del 128-194 | 68 | deletion in V1 and V2 regions; Figure 55 |

Alignments of the sequences presented in the above
25 tables are presented in Figures 66A and 66B.

A common region (Env-common) extends from nucleotide
position 1186 to nucleotide position 1329 (SEQ ID NO:69,

Fig. 56) relative to the wild-type US4 sequence and from nucleotide position 1117 to position 1260 (SEQ ID NO:79, Fig. 57) relative to the wild-type SF162 sequence. The synthetic sequences of the present invention

5 corresponding to these regions are presented, as SEQ ID NO:71 (Figure 58) for the synthetic Env US4 common region and as SEQ ID NO:72 (Figure 59) for the synthetic Env SF162 common region.

Percent identity to this sequence can be determined, 10 for example, using the Smith-Waterman search algorithm (Time Logic, Incline Village, NV), with the following exemplary parameters: weight matrix = nuc4x4hb; gap opening penalty = 20, gap extension penalty = 5, reporting threshold = 1; alignment threshold = 20.

15 Various forms of the different embodiments of the present invention (e.g., constructs) may be combined.

F. Cloning Synthetic Env Expression Cassettes of the Present Invention.

20 The synthetic DNA fragments encoding the Env polypeptides were typically cloned into the eucaryotic expression vectors described above for Gag, for example, pCMVKm2/pCMVlink (Figure 4), pCMV6a, pESN2dhfr (Figure 13A), pCMVIII (Figure 13B; alternately designated as the 25 pCMV-PL-E-dhfr/neo vector).

Exemplary designations for pCMVlink vectors containing synthetic expression cassettes of the present invention are as follows: pCMVlink.gp140.modSF162; pCMVlink.gp140.-modSF162.delV2; 30 pCMVlink.gp140.mut.modSF162; pCMVlink.gp140.mut.modSF162.delV2; pCMVKm2.gp140modUS4; pCMVKm2.gp140.modUS4.delV2; pCMVKm2.gp140.mut.modUS4; and, pCMVKm2.gp140.mut.modUS4.delV1/V2.

G. Generation of Synthetic Tat Expression Cassettes

Tat coding sequences have also been modified according to the teachings of the present specification. The wild type nucleotide sequence encoding tat from variant SF162 is presented in Figure 76 (SEQ ID NO:85). The corresponding wild-type amino acid sequence is presented in Figure 77 (SEQ ID NO:86). Figure 81 (SEQ ID NO:89) shows the nucleotide sequence encoding the amino terminal of the tat protein and the codon encoding cystein-22 is underlined. Other exemplary constructs encoding synthetic tat polypeptides are shown in Figures 78 and 79 (SEQ ID NOs:87 and 88). In one embodiment (SEQ ID NO:88), the cystein residue at position 22 is replaced by a glycine. Caputo et al. (1996) *Gene Therapy* 3:235 have shown that this mutation affects the trans activation domain of Tat.

Various forms of the different embodiments of the invention, described herein, may be combined.

H. Deposit of Vectors

Selected exemplary constructs shown below and described herein are deposited at Chiron Corporation, Emeryville, CA, 94662-8097, and were sent to the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on December 27, 1999.

| | Plasmid Name | Chiron Deposit # | Date Sent to ATCC |
|----|--------------------------------|---------------------|----------------------|
| | pCMVgpl60.modUS4 | 5094 | 27 Dec 99 |
| | pCMVgpl60delI.modUS4 | 5095 | 27 Dec 99 |
| | pCMVgpl60del2.modUS4 | 5096 | 27 Dec 99 |
| 5 | pCMVgpl60del-2.modUS4 | 5097 | 27 Dec 99 |
| | pCMVgpl60del128-194.mod.US4 | 5098 | 27 Dec 99 |
| | pCMVgpl40mut.modUS4del128-194 | 5100 | 27 Dec 99 |
| | pCMVgpl40.mut.mod.US | 5101 | 27 Dec 99 |
| | pCMVgpl60.modSF162 | 5125 | 27 Dec 99 |
| 10 | pCMVgpl60.modSF162.delV2 | 5126 | 27 Dec 99 |
| | pCMVgpl60.modSF162.delV1V2 | 5127 | 27 Dec 99 |
| | pCMVgpl40.mut.modSF162delV2 | 5128 | 27 Dec 99 |
| | pCMVgpl40.mut7.modSF162 | 5129 | 27 Dec 99 |
| | pCMVgpl40.mut7.modSF162delV2 | 5130 | 27 Dec 99 |
| 15 | pCMVgpl40.mut8.modSF162 | 5131 | 27 Dec 99 |
| | pCMVgpl40.mut8.modSF162delV2 | 5132 | 27 Dec 99 |
| | pCMVgpl40.mut8.modSF162delV1V2 | 5133 | 27 Dec 99 |
| | pCMVKm2.Gagprot.Mod.SF2.GP1 | 5150 | 27 Dec 99 |
| 20 | pCMVKm2.Gagprot.Mod.SF2.GP2 | 5151 | 27 Dec 99 |

Example 2

Expression Assays for the

Synthetic Gag, Env and Tat Coding Sequences

25 A. Gag and Gag-Protease Coding Sequences

The HIV-1SF2 wild-type Gag (SEQ ID NO:1) and Gag-protease (SEQ ID NO:2) sequences were cloned into expression vectors having the same features as the vectors into which the synthetic Gag (SEQ ID NO:4) and
 30 Gag-protease (SEQ ID NOs:5, 78 or 79)) sequences were cloned.

Expression efficiencies for various vectors carrying the HIV-1SF2 wild-type and synthetic Gag sequences were evaluated as follows. Cells from several mammalian cell lines (293, RD, COS-7, and CHO; all obtained from the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209) were transfected with 2 µg of DNA in transfection reagent LT1 (PanVera Corporation, 545 Science Dr., Madison, WI). The cells were incubated for 5 hours in reduced serum medium (Opti-MEM, Gibco-BRL, Gaithersburg, MD). The medium was then replaced with normal medium as follows: 293 cells, IMDM, 10% fetal calf serum, 2% glutamine (BioWhittaker, Walkersville, MD); RD and COS-7 cells, D-MEM, 10% fetal calf serum, 2% glutamine (Opti-MEM, Gibco-BRL, Gaithersburg, MD); and CHO cells, Ham's F-12, 10% fetal calf serum, 2% glutamine (Opti-MEM, Gibco-BRL, Gaithersburg, MD). The cells were incubated for either 48 or 60 hours. Supernatants were harvested and filtered through 0.45 µm syringe filters and, optionally, stored at -20°C.

Supernatants were evaluated using the Coulter p24-assay (Coulter Corporation, Hialeah, FL, US), using 96-well plates coated with a murine monoclonal antibody directed against HIV core antigen. The HIV-1 p24 antigen binds to the coated wells. Biotinylated antibodies against HIV recognize the bound p24 antigen. Conjugated streptavidin-horseradish peroxidase reacts with the biotin. Color develops from the reaction of peroxidase with TMB substrate. The reaction is terminated by addition of 4N H₂SO₄. The intensity of the color is directly proportional to the amount of HIV p24 antigen in a sample.

The results of these expression assays are presented in Tables 2A and 2B. Tables 2A and 2B shows data

obtained using the synthetic Gag-protease expression cassette of SEQ ID NO:5. Similar results were obtained using the Gag-protease expression cassettes of SEQ ID NOs:78 and 79.

5

Table 2: in vitro gag and gagprot p24 expression

5 TABLE 2a. Increased in vitro expression from modified vs. native gag plasmids in supernatants and lysates from transiently transfected cells

| experiment | native (nat) ^a modified (mod) ^b | supernatant (sup) lysate (lys) | cell line | hours post transfection | total ng p24 (fold increase) |
|------------|--|-----------------------------------|-----------|----------------------------|---------------------------------|
| 1 | nat | sup | 293 | 48 | 3.4 |
| | mod | sup | 293 | 48 | 1260 (371) |
| | nat | sup | 293 | 60 | 3.2 |
| | mod | sup | 293 | 60 | 2222 (694) |
| 2 | nat | sup | 293 | 60 | 1.8 |
| | mod | sup | 293 | 60 | 1740 (966) |
| 3 | nat | sup | 293 | 60 | 1.8 |
| | mod | sup | 293 | 60 | 580 (322) |
| 4 | nat | lys | 293 | 60 | 1.5 |
| | mod | lys | 293 | 60 | 85 (57) |
| 1 | nat | sup | RD | 48 | 5.6 |
| | mod | sup | RD | 48 | 66 (12) |
| | nat | sup | RD | 60 | 7.8 |
| | mod | sup | RD | 60 | 70.2 (9) |
| 2 | nat | lys | RD | 60 | 1.9 |
| | mod | lys | RD | 60 | 7.8 (4) |
| 1 | nat | sup | COS-7 | 48 | 0.4 |
| | mod | sup | COS-7 | 48 | 33.4 (84) |
| 2 | nat | sup | COS-7 | 48 | 0.4 |
| | mod | sup | COS-7 | 48 | 10 (25) |
| | nat | lys | COS-7 | 48 | 3 |
| | mod | lys | COS-7 | 48 | 14 (5) |

^a pCMVLink.Gag.SF2.PRE

^b pCMVKm2.GagMod.SF2

5 TABLE 2b. *In vitro* expression from modified *gag* and *gagprotease* plasmids in supernatants and lysates from transiently transfected cells

| plasmid | supernatant (sup) lysate (lys) | cell line | hours post transfection | total ng p24 ^d |
|---------------------------|-----------------------------------|-----------|----------------------------|---------------------------|
| Gag ^a | sup | 293 | 60 | 760 |
| GagProt(GP1) ^b | sup | 293 | 60 | 380 |
| GagProt(GP2) ^c | sup | 293 | 60 | 320 |
| Gag | lys | 293 | 60 | 78 |
| GagProt(GP1) | lys | 293 | 60 | 1250 |
| GagProt(GP2) | lys | 293 | 60 | 400 |
| Gag | sup | COS-7 | 72 | 40 |
| GagProt(GP1) | sup | COS-7 | 72 | 150 |
| GagProt(GP2) | sup | COS-7 | 72 | 290 |
| Gag | lys | COS-7 | 72 | 60 |
| GagProt(GP1) | lys | COS-7 | 72 | 63 |
| GagProt(GP2) | lys | COS-7 | 72 | 58 |

^a pCMVKm2.GagMod.SF2

^b pCMVKm2.GagProtMod.SF2(GP1) *gagprotease* with codon optimization and inactivation of INS in *protease*

^c pCMVKm2.GagProtMod.SF2(GP2) *gagprotease* with only inactivation of INS in *protease*

^d Shown are representative results from 3 independent experiments for each cell line tested.

The data showed that the synthetic Gag and Gag-protease expression cassettes provided dramatic increases in production of their protein products, relative to the native (HIV-1SF2 wild-type) sequences, when expressed in a variety of cell lines.

B. Env Coding Sequences

The HIV-SF162 ("SF162") wild-type Env (SEQ ID NO:1-3) and HIV-US4 ("US4") wild-type Env (SEQ ID NO:22-24) sequences were cloned into expression vectors having the same features as the vectors into which the synthetic Env sequences were cloned.

Expression efficiencies for various vectors carrying the SF162 and US4 wild-type and synthetic Env sequences were evaluated essentially as described above for Gag except that cell lysates were prepared in 40 μ l lysis buffer (1.0 % NP40, 0.1 M Tris pH 7.5) and frozen at -20°C and capture ELISAs were performed as follows.

For Capture ELISAs, 250 ng of an ammonium sulfate IgG cut of goat polyclonal antibody to gp120SF2/env2-3 was used to coat each well of a 96-well plate (Corning, Corning, NY). Serial dilutions of gp120/SF2 protein (MID 167) were used to set the quantitation curve from which expression of US4 or SF162 gp120 proteins from transfection supernatant and lysates were calculated. Samples were screened undiluted and, optionally, by serial 2-fold dilutions. A human polyclonal antibody to HIV-1 gp120/SF2 was used to detect bound gp120 envelope protein, followed by horse-radish peroxidase (HRP)-labeled goat anti-human IgG conjugates. TMB (Pierce, Rockford, IL) was used as the substrate and the reaction is terminated by addition of 4N H₂SO₄. The reaction was quantified by measuring the optical density (OD) at 450 nm. The intensity of the color is directly

proportional to the amount of HIV gp120 antigen in a sample. Purified SF2 gp120 protein was diluted and used as a standard.

5 The results of the transient expression assays are presented in Tables 3 and 4. Table 3 depicts transient expression in 293 cells transfected with a pCMVKm2 vector carrying the Env cassette of interest. Table 4 depicts transient expression in RD cells transfected with a pCMVKm2 vector carrying the Env cassette of interest.

5

Table 3

| Native (N) Synthetic (S) | Cell Line | Total sup (ng) | Sup fold increase (S v. N) | Total cell lysate (ng) | Cell lysate fold increase (S v. N) | Total (ng) | Total fold increase (S v. N) |
|-----------------------------|--------------|----------------------|----------------------------------|------------------------------|--|---------------|------------------------------------|
| N-gp120.US4 | RD | 87 | | <1 | | 88 | |
| S-gp120.modUS4 | RD | 690 | 8 | 2 | 5 | 693 | 8 |
| N-gp140.US4 | RD | 526 | | 0 | | 526 | |
| S-gp140.modUS4 | RD | 1305 | 2 | 1 | 2 | 1306 | 2 |
| S-gp140mut.modUS4 | RD | 35 | N/A | 25 | N/A | 60 | N/A |
| S-gp140TM.modUS4 | RD | 0 | N/A | 5 | N/A | 5 | N/A |
| N-gp160.US4 | RD | 0 | | 8 | | 8 | |
| S-gp160.modUS4 | RD | 0 | 0 | 30 | 4 | 30 | 4 |

Table 4

| CHO Cell Lines Expression Level of US4 Envelope Constructs | | | |
|---|-------------|--------------|------------------------------|
| Constructs | CHO Clone # | MTX Level | Expression Level* (ng/ml) |
| 5 gp120.modUS4 | 1 | 3.2 μ M | 250-450 |
| | 2 | 1.6 μ M | 350-450 |
| | 3 | 200nM | 230-580 |
| | 4 | 200nM | 300-500 |
| gp140.modUS4 | 1 | 1 μ M | 155-300 |
| | 2 | 1 μ M | 100-260 |
| | 3 | 1 μ M | 200-430 |
| gp140.mut. modUS4 | 1 | 1 μ M | 110-270 |
| | 2 | 1 μ M | 100-235 |
| | 3 | 1 μ M | 100-220 |
| 10 gp140.modUS4 .delV1/V2 | 1 | 50nM | 313-587** |
| | 2 | 50nM | 237-667** |
| | 3 | 50nM | 492-527** |
| gp140.mut. modUS4.delV1 /V2 | 1 | 50nM | 46-328** |
| | 2 | 50nM | 82-318** |
| | 3 | 50nM | 204-385** |

*All samples measured at T-75 flask stage unless otherwise indicated

**at 24 well and 6 well plate stages

***in a three liter bioreactor perfusion culture this clone yielded approximately 2-5 μ g/ml.

The data showed that the synthetic Env and expression cassettes provided a significant increase in production of their protein products, relative to the native (HIV-1SF162 or US4 wild-type) sequences, when
5 expressed in a variety of cell lines.

C. CHO Cell line Env expression data

Chinese hamster ovary (CHO) cells were transfected with plasmid DNA encoding the synthetic HIV-1 gp120 or
10 gp140 proteins (e.g., pESN2dhfr or pCMVIII vector backbone) using Mirus TransIT-LT1 polyamine transfection reagent (Pan Vera) according to the manufacturers instructions and incubated for 96 hours. After 96 hours, media was changed to selective media (F12 special with
15 250 µg/ml G418) and cells were split 1:5 and incubated for an additional 48 hours. Media was changed every 5-7 days until colonies started forming at which time the colonies were picked, plated into 96 well plates and screened by gp120 Capture ELISA. Positive clones were
20 expanded in 24 well plates and screened several times for Env protein production by Capture ELISA, as described above. After reaching confluency in 24 well plates, positive clones were expanded to T25 flasks (Corning, Corning, NY). These were screened several times after
25 confluency and positive clones were expanded to T75 flasks.

Positive T75 clones were frozen in LN2 and the highest expressing clones amplified with 0-5 µM methotrexate (MTX) at several concentrations and plated in
30 100mm culture dishes. Plates were screened for colony formation and all positive clones were again expanded as described above. Clones were expanded and amplified and screened at each step by gp120 capture ELISA. Positive clones were frozen at each methotrexate level. Highest

producing clones were grown in perfusion bioreactors (3L, 100L) for expansion and adaptation to low serum suspension culture conditions for scale-up to larger bioreactors.

- 5 Tables 5 and 6 show Capture ELISA data from CHO cells transfected with pCMVIII vector carrying a cassette encoding synthetic HIV-US4 and SF162 Env polypeptides (e.g., mutated cleavage sites, modified codon usage and/or deleted hypervariable regions). Thus, stably
- 10 transfected CHO cell lines which express Env polypeptides (e.g., gp120, gp140-monomeric, and gp140-oligomeric) have been produced.

Table 5

| CHO Cell Lines Expression Level of US4 Envelope Constructs | | | |
|---|-------------|--------------|-----------------------------|
| Constructs | CHO Clone # | MTX Level | Expression Level (ng/ml) |
| gp120.modUS4 | 1 | 3.2 μ M | 250-450 |
| | 2 | 1.6 μ M | 350-450 |
| | 3 | 200nM | 230-580*** |
| | 4 | 200nM | 300-500 |
| gp140.modUS4 | 1 | 1 μ M | 155-300 |
| | 2 | 1 μ M | 100-260 |
| | 3 | 1 μ M | 200-430 |
| gp140.mut. modUS4 | 1 | 1 μ M | 110-270 |
| | 2 | 1 μ M | 100-235 |
| | 3 | 1 μ M | 100-220 |
| gp140.modUS4 .delV1/V2 | 1 | 50nM | 313-587** |
| | 2 | 50nM | 237-667** |
| | 3 | 50nM | 492-527** |
| gp140.mut. modUS4.delV1 /V2 | 1 | 50nM | 46-328** |
| | 2 | 50nM | 82-318** |
| | 3 | 50nM | 204-385** |

*All samples measured at T-75 flask stage unless otherwise indicated

**at 24 well and 6 well plate stages

***in a three liter bioreactor perfusion culture this clone yielded approximately 2-5 μ g/ml.

Table 6

| CHO Cell Lines Expression Level of SF162 Envelope Constructs | | | |
|---|-------------|--------------|------------------------------|
| Constructs | CHO Clone # | MTX Level | Expression Level* (ng/ml) |
| gp120.modSF162 | 1 | 0 | 755-2705 |
| | 2 | 0 | 928-1538 |
| | 3 | 0 | 538-1609 |
| gp140.modSF162 | 1 | 20 nM | 180-350 |
| gp140.mut. modSF162 | 1 | 20 nM | 164-451 |
| | 2 | 20 nM | 188-487 |
| | 3 | 20 nM | 233-804 |
| gp120.modSF162 .delV2 | 1 | 800nM | 528-1560 |
| | 2 | 800nM | 487-1878 |
| | 3 | 800nM | 589-1212 |
| gp140.modSF162 .delV2 | 1 | 800nM | 300-600 |
| | 2 | 800nM | 200-400 |
| | 3 | 800nM | 200-500 |
| gp140.mut. modSF162.delV2 | 1 | 800nM | 300-700 |
| | 2 | 400nM | 1161 |
| | 3 | 800nM | 400-600 |
| | 4 | 400nM | 1600-2176 |

*All samples measured at T-75 flask stage unless otherwise indicated

The results presented above demonstrate the ability of the constructs of the present invention to provide expression of Env polypeptides in CHO cells. Production of polypeptides using CHO cells provides (i) correct glycosylation patterns and protein conformation (as determined by binding to panel of MAbs); (ii) correct binding to CD4 receptor molecules; (iii) absence of non-

mammalian cell contaminants (e.g., insect viruses and/or cells); and (iv) ease of purification.

D. Tat Coding Sequences

5 The HIV-SF162 ("SF162") wild-type Tat (SEQ ID NO:85) sequences were cloned into expression vectors having the same features as the vectors into which the synthetic Tat sequences were cloned (SEQ ID NOs:87, 88 and 89).

10 Expression efficiencies for various vectors carrying the SF162 wild-type and synthetic Tat sequences are evaluated essentially as described above for Gag and Env using capture ELISAs with the appropriate anti-tat antibodies and/or CHO cell assays. Expression of the polypeptides encoded by the synthetic cassettes is
15 improved relative to wild type.

Example 3

Western Blot Analysis of Expression

A. Gag and Gag-Protease Coding Sequences

20 Human 293 cells were transfected as described in Example 2 with pCMV6a-based vectors containing native or synthetic Gag expression cassettes. Cells were cultivated for 60 hours post-transfection. Supernatants were prepared as described. Cell lysates were prepared
25 as follows. The cells were washed once with phosphate-buffered saline, lysed with detergent [1% NP40 (Sigma Chemical Co., St. Louis, MO) in 0.1 M Tris-HCl, pH 7.5], and the lysate transferred into fresh tubes. SDS-polyacrylamide gels (pre-cast 8-16%; Novex, San Diego,
30 CA) were loaded with 20 μ l of supernatant or 12.5 μ l of cell lysate. A protein standard was also loaded (5 μ l, broad size range standard; BioRad Laboratories, Hercules, CA). Electrophoresis was carried out and the proteins were transferred using a BioRad Transfer Chamber (BioRad

Laboratories, Hercules, CA) to Immobilon P membranes (Millipore Corp., Bedford, MA) using the transfer buffer recommended by the manufacturer (Millipore), where the transfer was performed at 100 volts for 90 minutes. The membranes were exposed to HIV-1-positive human patient serum and immunostained using o-phenylenediamine dihydrochloride (OPD; Sigma).

The results of the immunoblotting analysis showed that cells containing the synthetic Gag expression cassette produced the expected p55 protein at higher per-cell concentrations than cells containing the native expression cassette. The Gag p55 protein was seen in both cell lysates and supernatants. The levels of production were significantly higher in cell supernatants for cells transfected with the synthetic Gag expression cassette of the present invention. Experiments performed in support of the present invention suggest that cells containing the synthetic Gag-prot expression cassette produced the expected Gag-prot protein at comparably higher per-cell concentrations than cells containing the native expression cassette.

In addition, supernatants from the transfected 293 cells were fractionated on sucrose gradients. Aliquots of the supernatant were transferred to Polyclear™ ultra-centrifuge tubes (Beckman Instruments, Columbia, MD), under-laid with a solution of 20% (wt/wt) sucrose, and subjected to 2 hours centrifugation at 28,000 rpm in a Beckman SW28 rotor. The resulting pellet was suspended in PBS and layered onto a 20-60% (wt/wt) sucrose gradient and subjected to 2 hours centrifugation at 40,000 rpm in a Beckman SW41ti rotor.

The gradient was then fractionated into approximately 10 x 1 ml aliquots (starting at the top, 20%-end, of the gradient). Samples were taken from

fractions 1-9 and were electrophoresed on 8-16% SDS polyacrylamide gels. Fraction number 4 (the peak fraction) corresponds to the expected density of Gag protein VLPs. The supernatants from 293/synthetic Gag cells gave much stronger p55 bands than supernatants from 293/native Gag cells, and, as expected, the highest concentration of p55 in either supernatant was found in fraction 4.

These results demonstrate that the synthetic Gag expression cassette provides superior production of both p55 protein and VLPs, relative to the native Gag coding sequences.

B. Env Coding Sequences

Human 293 cells were transfected as described in Example 2 with pCMVKm2-based; pCMVlink-based; p-CMVII-based or pESN2-based vectors containing native or synthetic Env expression cassettes. Cells were cultivated for 48 or 60 hours post-transfection. Cell lysates and supernatants were prepared as described (Example 2). Briefly, the cells were washed once with phosphate-buffered saline, lysed with detergent [1% NP40 (Sigma Chemical Co., St. Louis, MO)] in 0.1 M Tris-HCl, pH 7.5], and the lysate transferred into fresh tubes. SDS-polyacrylamide gels (pre-cast 8-16%; Novex, San Diego, CA) were loaded with 20 μ l of supernatant or 12.5 μ l of cell lysate. A protein molecular weight standard and an HIV SF2 gp120 positive control protein (5 μ l, broad size range standard; BioRad Laboratories, Hercules, CA) were also loaded. Electrophoresis was carried out and the proteins were transferred using a BioRad Transfer Chamber (BioRad Laboratories, Hercules, CA) to Immobilon P membranes (Millipore Corp., Bedford, MA) using the transfer buffer recommended by the manufacturer

(Millipore), where the transfer was performed at 100 volts for 90 minutes. The membranes were then reacted against polyclonal goat anti-gp120SF2/env2-3 anti-sera, followed by incubation with swine anti-goat IgG-
5 peroxidase (POD) (Sigma, St. Louis, MO). Bands indicative of binding were visualized by adding DAB with hydrogen peroxide which deposits a brown precipitate on the membranes.

The results of the immunoblotting analysis showed
10 that cells containing the synthetic Env expression cassette produced the expected Env gp proteins of the predicted molecular weights as determined by mobilities in SDS-polyacrylamide gels at higher per-cell concentrations than cells containing the native
15 expression cassette. The Env proteins were seen in both cell lysates and supernatants. The levels of production were significantly higher in cell supernatants for cells transfected with the synthetic Env expression cassette of the present invention.

20 C. Tat Coding Sequences

Human 293 cells are transfected as described in Example 2 with various vectors containing native or synthetic Tat expression cassettes. Cells are cultivated
25 and isolated proteins analyzed as described above. Immunoblotting analysis shows that cells containing the synthetic Tat expression cassette produced the expected Tat proteins of the predicted molecular weights as determined by mobilities in SDS-polyacrylamide gels at
30 higher per-cell concentrations than cells containing the native expression cassette.

Example 4Purification of Env polypeptidesA. Purification of Oligomeric gp140

Purification of oligomeric gp140 (o-gp140 US4) was
5 conducted essentially as shown in Figure 60. For the
experiments described herein, o-gp140 refers to
oligomeric gp140 in either native or modified (e.g.,
optimized expression sequences, deleted, mutated,
truncated, etc.) form. Briefly, concentrated (30-50X)
10 supernatants obtained from CHO cell cultures were loaded
onto an anion exchange (DEAE) column which removed DNA
and other serum proteins. The eluted material was loaded
onto a ceramic hydroxyapatite column (CHAP) which bound
serum proteins but not HIV Env proteins. The flow-
15 through from the DEAE and CHAP columns was loaded onto a
Protein A column as a precautionary step to remove any
remaining serum immunoglobulins. The Env proteins in the
flow-through were then captured using the lectin
gluconanthus navalis (GNA, Vector Labs, Burlingame, CA).
20 GNA has high affinity for mannose rich carbohydrates such
as Env. The Env proteins were then eluted with GNA
substrate. To remove other highly glycosylated proteins,
a cation exchange column (SP) was used to purify
gp140/gp120. In a final step, which separates gp120 from
25 o-gp140, a gel filtration column was used to separate
oligomers from monomers. Sizing and chromatography
analysis of the final product revealed that this strategy
lead to the successful isolation of oligomeric gp140.

B. Purification of gp120

30 Purification of gp120 was conducted essentially as
previously described for other Env proteins. Briefly,
concentrated supernatants obtained from CHO cell cultures
were loaded onto an anion exchange (DEAE) column which

removed DNA and other serum proteins. The eluted material was loaded onto a ceramic hydroxyapatite column (CHAP) which bound serum proteins but not HIV Env proteins. The flow-through from the CHAP column was
5 loaded a cation exchange column (SP) where the flow-through was discarded and the bound fraction eluted with salt. The eluted fraction(s) were loaded onto a Suprose 12/Superdex 200 Tandem column (Pharmacia-Upjohn, Uppsala, Sweden) from which purified gp120 was obtained. Sizing
10 and chromatography analysis of the final product revealed that this strategy successfully purified gp120 proteins.

Example 5

Analysis of Purified Env Polypeptides

15 A. Analysis of o-gp140

It is well documented that HIV Env protein binds to CD4 only in its correct conformation. Accordingly, the ability of o-gp140 US4 polypeptides, produced and purified as described above, to bind CD4 cells was
20 tested. O-gp140 US4 was incubated for 15 minutes with FITC-labeled CD4 at room temperature and loaded onto a Biosil 250 (BioRad) size exclusion column using Waters HPLC. CD4-FITC has the longest retention time (2.67 minutes), followed by CD4-FITC-gp120 (2.167 min). The
25 shortest retention time (1.9 min) was observed for CD4-FITC-o-gp140 US4 indicating that, as expected, o-gp140 US4 binds to CD4 forming a large complex which reduces retention time on the column. Thus, the o-gp140 US4 produced and purified as described above is of the
30 correct size and conformation.

In addition, the US4 o-gp140, purified as described above, was also tested for its ability to bind to a variety of monoclonal antibodies with known epitope specificities for the CD4 binding site, the CD4 inducible

site, the V3 loop and oligomer-specific gp41 epitope. O-gp140 bound strongly to these antibodies, indicating that the purified protein retains its structural integrity.

5 B. Analysis of gp120

As described above, CD4-FITC binds gp120, as demonstrated by the decreased retention time on the HPLC column. Thus, US4 gp120 purified by the above method retains its conformational integrity. In addition, the properties of purified gp120 can be tested by examining its integrity and identity on western blots, as well as, by examining protein concentration, pH, conductivity, endotoxin levels, bioburden and the like. US4 gp120, purified as described above, was also tested for its ability to bind to a variety of monoclonal antibodies with known epitope specificities for the CD4 binding site, the CD4 inducible site, the V3 loop and oligomer-specific gp41 epitope. The pattern of mAb binding to gp120 indicated that the purified protein retained its structural integrity, for example, the purified gp120 did not bind the mAb having the oligomer-specific gp41 epitope (as expected).

Example 6

25 Electron Microscopic Evaluation of VLP Production

The cells for electron microscopy were plated at a density of 50-70% confluence, one day before transfection. The cells were transfected with 10 µg of DNA using transfection reagent LT1 (Panvera) and incubated for 5 hours in serum-reduced medium (see Example 2). The medium was then replaced with normal medium (see Example 2) and the cells were incubated for 14 hours (COS-7) or 40 hours (CHO). After incubation the cells were washed twice with PBS and fixed with 2%

glutaraldehyde. Electron microscopy was performed by Prof. T.S. Benedict Yen, Veterans Affairs, Medical Center, San Francisco, CA).

Electron microscopy was carried out using a transmission electron microscope (Zeiss 10c). The cells were pre-stained with osmium and stained with uranium acetate and lead citrate. The magnification was 100,000X.

Figures 3A and 3B show micrographs of CHO cells transfected with pCMVKM2 carrying the synthetic Gag expression cassette (SEQ ID NO:5) or carrying the Gag-prot expression cassette (SEQ ID NO:79). In the figure, free and budding immature virus-like-particles (VLP) of the expected size (100 nm) are seen for the Gag expression cassette (Figure 3A) and both immature and mature VLPs are seen for the Gag-prot expression cassette (Figure 3B). COS-7 cells transfected with the same vector have the same expression pattern. VLP can also be found intracellularly in CHO and COS-7 cells.

Native and synthetic Gag expression cassettes were compared for their associated levels of VLP production when used to transfect human 293 cells. The comparison was performed by density gradient ultracentrifugation of cell supernatants and Western-blot analysis of the gradient fractions. There was a clear improvement in production of VLPs when using the synthetic Gag construct.

Example 7

Expression of Virus-like Particles in the Baculovirus System

A. Expression of Native HIV p55 Gag

To construct the native HIV p55 Gag baculovirus shuttle vector, the prototype SF2 HIV p55 plasmid, pTM1-

Gag (Selby M.J., et al., *J Virol.* 71(10):7827-7831, 1997), was digested with restriction endonucleases NcoI and BamHI to extract a 1.5 Kb fragment that was subsequently subcloned into pAcC4 (*Bio/Technology* 6:47-55, 1988), a derivative of pAc436. Generation of the recombinant baculovirus was achieved by co-transfecting 2 µg of the HIV p55 Gag pAcC4 shuttle vector with 0.5 µg of linearized, *Autographa californica* baculovirus (AcNPV) wild-type viral DNA into *Spodoptera frugiperda* (Sf9) cells (Kitts, P.A., Ayres M.D., and Possee R.D., *Nucleic Acids Res.* 18:5667-5672, 1990). The isolation of recombinant virus expressing HIV p55 Gag was performed according to standard techniques (O'Reilly, D.R., L.K. Miller, and V. A. Luckow, *Baculovirus Expression Vector: A Laboratory Manual*, W.H. Freeman and Company, New York, 1992).

Expression of the HIV p55 Gag was achieved using a 500 ml suspension culture of Sf9 cells grown in serum-free medium (Miaorella, B., D. Inlow, A. Shauger, and D. Harano, *Bio/Technology* 6:1506-1510, 1988) that had been infected with the HIV p55 Gag recombinant baculovirus at a multiplicity of infection (MOI) of 10. Forty-eight hours post-infection, the supernatant was separated by centrifugation and filtered through a 0.2 µm filter. Aliquots of the supernatant were then transferred to Polyclear™ (Beckman Instruments, Palo Alto, CA) ultracentrifuge tubes, underlaid with 20% (wt/wt) sucrose, and subjected to 2 hours centrifugation at 24,000 rpm using a Beckman SW28 rotor.

The resulting pellet was suspended in Tris buffer (20 mM Tris HCl, pH 7.5, 250 mM NaCl, and 2.5 mM ethylenediaminetetraacetic acid [EDTA]), layered onto a 20-60% (wt/wt) sucrose gradient, and subjected to 2 hours centrifugation at 40,000 rpm using a Beckman SW41ti

rotor. The gradient was then fractionated starting at the top (20% sucrose) of the gradient into approximately twelve 0.75 ml aliquots. A sample of each fraction was electrophoresed on 8-16% SDS polyacrylamide gels and the resulting bands were visualized after commassie staining (Figure 4). Additional aliquots were subjected to refractive index analysis.

The results shown in Figure 4 indicated that the p55 Gag virus-like particles banded at a sucrose density of range of 1.15 - 1.19 g/ml with the peak at approximately 1.17 g/ml. The peak fractions were pooled and concentrated by a second 20% sucrose pelleting. The resulting pellet was suspended in 1 ml of Tris buffer (described above). The total protein yield as estimated by Bicimchrominic Acid (BCA) (Pierce Chemical, Rockford, IL) was 1.6 mg.

B. Expression of Synthetic HIV p55 Gag

A baculovirus shuttle vector containing the synthetic p55 Gag sequence was constructed as follows. The synthetic HIV p55 expression cassette (Example 1) was digested with restriction enzyme *SalI* followed by incubation with T4-DNA polymerase. The resulting fragment was isolated (PCR Clean-Up™, Promega, Madison, WI) and then digested with *BamHI* endonuclease. The shuttle vector pAcC13 (Munemitsu S., et al., *Mol Cell Biol.* 10(11):5977-5982, 1990) was linearized by digestion with *EcoI*, followed by incubation with T4-DNA polymerase, and then isolated (PCR Clean-Up™). The linearized vector was digested with *BamHI*, treated with alkaline phosphatase, and isolated by size fragmentation in an agarose gel. The isolated 1.5 kb fragment was ligated with the prepared pAcC13 vector. The resulting clone was designated pAcC13-Modif.p55Gag.

The expression conditions for the synthetic HIV p55 VLPs differed from those of the native p55 Gag as follows: a culture volume of 1 liter used instead of 500 ml; *Trichoplusia ni* (Tn5) (Wickham, T.J., and Nermerow, G.R., *BioTechnology Progress*, 9:25-30, 1993) insect cells were used instead of Sf9 insect cells; and, an MOI of 3 was instead of an MOI of 10. Experiments performed in support of the present invention showed that there was no appreciable difference in expression level between the Sf9 and Tn5 insect cells with the native p55 clone. In terms of MOI, experience with the native p55 clone suggested that an MOI of 10 resulted in higher expression (approximately 2-fold) of VLPs than a lower MOI.

The sucrose pelleting and banding methods used for the synthetic p55 VLPs were similar to those employed for the native p55 VLPs (described above), with the following exceptions: pelleted VLPs were suspended in 4 ml of phosphate buffered saline (PBS) instead of 1.0 ml of the Tris buffer; and four, 20-60% sucrose gradients were used instead of a single gradient. Also, due to the high concentration of banded VLPs, further concentration by pelleting was not required. The peak fractions from all 4 gradients were simply dialyzed against PBS. The approximate density of the banded VLPs ranged from 1.23-1.28 g/ml. A total protein yield as estimated by BCA was 46 mg. Results from the sucrose gradient banding of the synthetic p55 are shown in Figure 5.

A comparison of the total amount of purified HIV p55 Gag from several preparations obtained from the two baculovirus expression cassettes has been summarized in Figure 6. The average yield from the native p55 was 3.16 mg/liter of culture (n=5, standard deviation (sd) ± 1.07 , range = 1.8-4.8 mg/L) whereas the average yield from the

synthetic p55 was more than ten-fold higher at 44.5 mg/liter of culture (n=2, sd=±6.4).

In addition to a higher total protein yield, the final product from the synthetic p55-expressed Gag consistently contained lower amounts of contaminating baculovirus proteins than the final product from the native p55-expressed Gag. This difference can be seen in the two commassie-stained gels Figures 4 and 5.

10 C. Expression of Native and Synthetic Gag-Core

Expression of the HIV p55 Gag/HCV Core 173 (SEQ ID NO:8) was achieved using a 2.5 liter suspension culture of Sf9 cells grown in serum-free medium (Miaorella, B., D. Inlow, A. Shauger, and D. Harano. 1988 Bio/Technology 6:1506-1510). The cells were infected with an HIV p55 Gag/HCV Core 173 recombinant baculovirus. Forty-eight hours post-infection, the supernatant was separated from the cells by centrifugation and filtered through a 0.2 μ m filter. Aliquots of the supernatant were then transferred to a Polyclear™ (Beckman Instruments, Palo Alto, CA) ultracentrifuge tubes containing 30% (wt/wt) sucrose, and subjected to 2 hours of centrifugation at 24,000 rpm in a Beckman SW28 rotor and ultracentrifuge.

The resulting pellet was suspended in Tris buffer (50 mM Tris-HCl, pH 7.5, 500 mM NaCl) and layered onto a 30-60% (wt/wt) sucrose gradient and subjected to 2 hours centrifugation at 40,000 rpm in a Beckman SW41ti rotor and ultracentrifuge. The gradient was then fractionated starting at the top (30%) of the gradient into approximately 11 x 1.0 ml aliquots. A sample of each fraction was electrophoresed on 8-16% SDS polyacrylamide gels and the resulting bands were visualized after commassie staining.

A subset of aliquots were also subjected to Western blot analysis using monoclonal antibody 76C.5EG (Steimer, K.S., et al., Virology 150:283-290, 1986) which is specific for HIV p24 (a subunit of HIV p55). The peak fractions from the sucrose gradient were pooled and concentrated by a second 20% sucrose pelleting. The resulting pellet was suspended in 1 ml of buffer Tris buffer and the total protein yield as estimated by BCA (Pierce Chemical, Rockford, IL) was ~ 1.0 mg.

The results from the SDS PAGE are shown in Figure 8 and the anti- p24 Western blot results are shown in Figure 9. Taken together, these results indicate that the HIV p55 Gag/HCV Core 173 chimeric VLPs banded at a sucrose density similar to that of the HIV p55 Gag VLPs and the visible protein band that migrated at a molecular weight of ~ 72,000 kd was reactive with the HIV p24-specific monoclonal antibody. An additional immunoreactive band at approximately 55,000 kd also appeared to be reactive with the anti-p24 antibody and may be a degradation product.

Although aliquots from the above preparation were not tested for reactivity with an HCV Core-specific antibody (an anti-CD22 rabbit serum), results from a similar preparation are shown in Figure 10 and indicate that the main HCV Core-specific reactivity migrates at an approximate molecular weight of 72,000 kd which is in accordance with the predicted molecular weight of the chimeric protein.

The expression conditions for the synthetic HIV p55 Gag/HCV Core 173 (SEQ ID NO:8) VLPs differed from those of the native p55 Gag and are as follows: a culture volume of 1 liter used instead of 2.5 liters, *Trichoplusia ni* (Tn5) (Wickham, T.J., and Nemerow, G.R. 1993 BioTechnology Progress, 9:25-30) insect cells were

used instead of Sf9 insect cells and an MOI of 3 was instead of an MOI of 10. The sucrose pelleting and banding methods used for the synthetic HIV p55 Gag/HCV Core 173 VLPs were similar to those employed for the native HIV p55 Gag/HCV Core 173 VLPs. However, differences included: pelleted VLPs were suspended in 1 ml of phosphate buffered saline (PBS) instead of 1.0 ml of the Tris buffer, and a single 20-60% sucrose gradients was used. A comparison of the total amount of purified HIV p55 Gag/HCV Core 173 from multiple preparations obtained from the two baculovirus expression cassettes showed that there was an increase in expression using the synthetic HIV p55 Gag/HCV Core 173 cassette.

D. Alternative method for the enrichment of HIV p55 Gag VLPs

In addition to purification from the media, p55 (Gag protein) expressed in baculovirus (e.g., using a synthetic expression cassette of the present invention) can also be purified as virus-like particles from the infected insect cells. For example, forty-eight hours post infection, the media and cell pellet are separated by centrifugation and the cell pellet is stored at -70°C until future use. At the time of processing, the cell pellet is suspended in 5 volumes of hypotonic lysis buffer (20 mM Tris-HCl, pH 8.2, 1 mM EGTA; 1 mM MgCl₂, and Complete Protease Inhibitor® (Boehringer Mannheim Corp., Indianapolis, IN)). If needed, the cells are then dounced 8-10 times to complete cell lysis.

The lysate is then centrifuged at approximately 1000-1500 x g for 20 minutes. The supernatant is

decanted into UltraClear™ tubes, underlayered with 20% sucrose (w/w) and centrifuged at 24,000 rpm in SW28 buckets for 2 hours. The resulting pellet is suspended in Tris buffer (20 mM Tris HCl, pH 7.5, 250 mM NaCl, and 2.5 mM ethylenediamine-tetraacetic acid (EDTA) with 0.1% IGEPAL detergent (Sigma Chemical, St. Louis, MO) and 250 units/ml of benzonase (American International Chemical, Inc., Natick, MA) and incubated at 4°C for at least 30 minutes. The suspension is subsequently layered onto a 20-60% sucrose gradient and spun at 40,000 rpm using an SW41ti rotor for 20-24 hours.

After ultracentrifugation, the sucrose gradient is fractionated and aliquots run on SDS PAGE to identify peak fractions. The peak fractions are dialyzed against PBS and measured for protein content. Negatively stained electron micrographs typically show non-enveloped VLPs somewhat smaller in diameter (80-120 nm) than the budded VLPs. HIV Gag VLPs prepared in this manner are also capable of generating Gag-specific CTL responses in mice.

Example 8

In Vivo Immunogenicity of Synthetic Gag Expression

Cassettes

A. Immunization

To evaluate the possibly improved immunogenicity of the synthetic Gag expression cassettes, a mouse study was performed. The plasmid DNA, pCMVKM2 carrying the synthetic Gag expression cassette, was diluted to the following final concentrations in a total injection volume of 100 µl: 20 µg, 2 µg, 0.2 µg, and 0.02 µg. To

overcome possible negative dilution effects of the diluted DNA, the total DNA concentration in each sample was brought up to 20 μg using the vector (pCMVKM2) alone. As a control, plasmid DNA of the native Gag expression cassette was handled in the same manner. Twelve groups of four Balb/c mice (Charles River, Boston, MA) were intramuscularly immunized (50 μl per leg, intramuscular injection into the *tibialis anterior*) according to the schedule in Table 7.

Table 7

| Group | Gag Expression Cassette | Concentration of Gag plasmid DNA (μg) | Immunized at time (weeks): |
|-------|-------------------------|--|----------------------------|
| 1 | Synthetic | 20 | 0 ¹ , 4 |
| 2 | Synthetic | 2 | 0, 4 |
| 3 | Synthetic | 0.2 | 0, 4 |
| 4 | Synthetic | 0.02 | 0, 4 |
| 5 | Synthetic | 20 | 0 |
| 6 | Synthetic | 2 | 0 |
| 7 | Synthetic | 0.2 | 0 |
| 8 | Synthetic | 0.02 | 0 |
| 9 | Native | 20 | 0 |
| 10 | Native | 2 | 0 |
| 11 | Native | 0.2 | 0 |
| 12 | Native | 0.02 | 0 |

1 = initial immunization at "week 0"

Groups 1-4 were bled at week 0 (before immunization), week 4, week 6, week 8, and week 12. Groups 5-12 were bled at week 0 (before immunization) and at week 4.

B. Humoral Immune Response

The humoral immune response was checked with an anti-HIV Gag antibody ELISAs (enzyme-linked immunosorbent assays) of the mice sera 0 and 4 weeks post immunization (groups 5-12) and, in addition, 6 and 8 weeks post immunization, respectively, 2 and 4 weeks post second immunization (groups 1-4).

The antibody titers of the sera were determined by anti-Gag antibody ELISA. Briefly, sera from immunized mice were screened for antibodies directed against the HIV p55 Gag protein. ELISA microtiter plates were coated with 0.2 μ g of HIV-1_{SP2} p24-Gag protein per well overnight and washed four times; subsequently, blocking was done with PBS-0.2% Tween (Sigma) for 2 hours. After removal of the blocking solution, 100 μ l of diluted mouse serum was added. Sera were tested at 1/25 dilutions and by serial 3-fold dilutions, thereafter. Microtiter plates were washed four times and incubated with a secondary, peroxidase-coupled anti-mouse IgG antibody (Pierce, Rockford, IL). ELISA plates were washed and 100 μ l of 3, 3', 5, 5'-tetramethyl benzidine (TMB; Pierce) was added per well. The optical density of each well was measured after 15 minutes. The titers reported are the reciprocal of the dilution of serum that gave a half-maximum optical density (O.D.). The ELISA results are presented in Table 8.

Table 8

| Group | Inoculum (μ g) | Expression cassette | Sera - Week 4 ³ | Sera - Week 6 | Sera - Week 8 |
|-------|------------------------|------------------------|-------------------------------|-------------------|------------------|
| 1 | 20 | S ¹ - gag | 98 | 455 | 551 |
| 2 | 2 | S - gag | 59 | 1408 | 227 |
| 3 | 0. | S - gag | 29 | 186 | 61 |
| 4 | 0.02 | S - gag | < 20 | < 20 | < 20 |
| 5 | 20 | S - gag | 67 | n.a. ⁴ | n.a. |
| 6 | 2 | S - gag | 63 | n.a. | n.a. |
| 7 | 0. | S - gag | 57 | n.a. | n.a. |
| 8 | 0.02 | S - gag | < 20 | n.a. | n.a. |
| 9 | 20 | N ² - gag | 43 | n.a. | n.a. |
| 10 | 2 | N - gag | < 20 | n.a. | n.a. |
| 11 | 0. | N - gag | < 20 | n.a. | n.a. |
| 12 | 0.02 | N - gag | < 20 | n.a. | n.a. |

1 = synthetic gag expression cassette (SEQ ID NO: 4)

2 = native gag expression cassette (SEQ ID NO: 1)

3 = geometric mean antibody titer

4 = not applicable

The results of the mouse immunizations with plasmid-DNAs show that the synthetic expression cassettes provide a clear improvement of immunogenicity relative to the native expression cassettes. Also, the second boost immunization induced a secondary immune response after two weeks (groups 1-3).

C. Cellular Immune Response

The frequency of specific cytotoxic T-lymphocytes (CTL) was evaluated by a standard chromium release assay of peptide pulsed Balb/c mouse CD4 cells. Gag expressing vaccinia virus infected CD-8 cells were used as a positive control (vvGag). Briefly, spleen cells (Effector cells, E) were obtained from the BALB/c mice immunized as described above (Table 8) were cultured, restimulated, and assayed for CTL activity against Gag

peptide-pulsed target cells as described (Doe, B., and Walker, C.M., *AIDS* 10(7):793-794, 1996). The HIV-1_{sf2} Gag peptide used was p7g SEQ ID NO:10. Cytotoxic activity was measured in a standard ⁵¹Cr release assay. Target (T)

5 cells were cultured with effector (E) cells at various E:T ratios for 4 hours and the average cpm from duplicate wells was used to calculate percent specific ⁵¹Cr release. The results are presented in Table 9.

Cytotoxic T-cell (CTL) activity was measured in
10 splenocytes recovered from the mice immunized with HIV Gag DNA (compare Effector column, Table 9, to immunization schedule, Table 8). Effector cells from the Gag DNA-immunized animals exhibited specific lysis of Gag p7g peptide-pulsed SV-BALB (MHC matched) targets cells
15 indicative of a CTL response. Target cells that were peptide-pulsed and derived from an MHC-unmatched mouse strain (MC57) were not lysed (Table 9; MC/p7g).

Table 9

| Table 9. Cytotoxic T-lymphocyte (CTL) responses in mice immunized with HIV-1 <i>gag</i> DNA | | | | |
|---|-------|---|------------|---------|
| Immunization | E:T | Percent specific lysis of target cells* | | |
| | | SVBALB none | SVBALB p7g | RMA p7g |
| 20 μ g DNA gagmod | 100:1 | 2 | 49 | <1 |
| | 30:1 | 3 | 30 | <1 |
| | 10:1 | <1 | 14 | <1 |
| 2 μ g DNA gagmod | 100:1 | 2 | 37 | <1 |
| | 30:1 | 2 | 21 | <1 |
| | 10:1 | <1 | 13 | <1 |
| 0.2 μ g DNA gagmod | 100:1 | 2 | 32 | <1 |
| | 30:1 | 3 | 25 | <1 |
| | 10:1 | 1 | 14 | <1 |
| 0.02 μ g DNA gagmod | 100:1 | 1 | 17 | <1 |
| | 30:1 | 1 | 16 | <1 |
| | 10:1 | 1 | 8 | <1 |
| 20 μ g DNA gag native | 100:1 | 2 | 49 | <1 |
| | 30:1 | 2 | 24 | <1 |
| | 10:1 | 1 | 12 | <1 |
| 2 μ g DNA gag native | 100:1 | <1 | 18 | <1 |
| | 30:1 | 1 | 14 | <1 |
| | 10:1 | 1 | 7 | <1 |
| 0.2 μ g DNA gag native | 100:1 | 3 | 30 | <1 |
| | 30:1 | 3 | 17 | <1 |
| | 10:1 | 2 | 7 | <1 |
| 0.02 μ g DNA gag native | 100:1 | 4 | 2 | <1 |
| | 30:1 | 1 | 2 | <1 |
| | 10:1 | 1 | 2 | <1 |

*representative results of two animals per DNA-dose; positive CTL responses are indicated by boxed data

The results of the CTL assays show increased potency of synthetic Gag expression cassettes for induction of cytotoxic T-lymphocyte (CTL) responses by DNA immunization.

Example 9In vivo Immunization with Env polypeptidesA. Immunogenicity Study of US4 o-gp140 in Ras-3c Adjuvant System

5 Studies have been conducted using rabbits immunized with US4 o-gp140 purified as described above. Studies are also underway in animals to determine immunogenicity of US4 gp120, SF162 o-gp140 and SF162 gp120.

10 Two rabbits (#1 and #2) were immunized intramuscularly at 0, 4, 12 and 24 weeks with 50 µg of US4 o-gp140 in the Ribi™ adjuvant system (RAS-3c), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL, Ribi Immunochem, Hamilton, MT).

15 In each experiment described herein, o-gp140 can be native, mutated and/or modified. Antibody responses directed against the US4 o-gp140 protein were measured by ELISA. Results are shown in Table 10.

Table 10

| Rabbit/sample | Approximate o-gp140 ELISA titer |
|-----------------------------|---------------------------------|
| pre-immunization | 0 |
| #1: post1 (0 week immuniz) | 400 |
| #1: post2 (4 week immuniz) | 15,000 |
| #1: post3 (12 week immuniz) | 50,000 |
| #1: post4 (24 week immuniz) | 100,000 |
| #2: post1 (0 week immuniz) | 600 |
| #2: post2 (4 week immuniz) | 12,000 |
| #2: post3 (12 week immuniz) | 25,000 |
| #2: post4 (24 week immuniz) | 55,000 |

The avidities of antibodies directed against the US4 o-gp140 protein were measured in a similar ELISA format employing successive washes with increasing concentrations of ammonium isothiocyanate. Results are shown in Table 11.

Table 11

| Time of sample | Approx. Antibody avidity (NH ₄ HCN Conc. in M) |
|-------------------------|--|
| pre-immunization | 0.02 |
| post1 (0 week immuniz) | 1.8 |
| post2 (4 week immuniz) | 3.5 |
| post3 (12 week immuniz) | 5.5 |
| post4 (24 week immuniz) | 5.1 |

These results show that US4 o-gp140 is highly immunogenic and able to induce substantial antibody responses after only one or two immunizations.

5 B. Immunogenicity of US4 o-gp140 in MF59-based Adjuvants

Groups of 4 rabbits were immunized intramuscularly at 0, 4, 12 and 24 weeks with various doses of US4 o-gp140 protein in three different MF59-based adjuvants (MF59 is described in International Publication No. WO 90/14837 and typically contains 5% Squalene, 0.5% Tween 80, and 0.5% Span 85). Antibody titers were measured post-third by ELISA using SF2 gp120 to coat the plates. QHC is a quill-based adjuvant (Iscotek, Uppsala, Sweden). Results are shown in Table 12.

Table 12

| Antigen dose (μ g) | Adjuvant | Anti-gp120 _{SF2} Ab GMT* |
|-------------------------|--------------|-----------------------------------|
| 12.5 | MF59 | 7231 |
| 25 | MF59 | 8896 |
| 50 | MF59 | 12822 |
| 12.5 | MF59/MPL | 24146 |
| 25 | MF59/MPL | 27199 |
| 50 | MF59/MPL | 23059 |
| 50 | MF59/MPL/QHC | 31759 |

*GMT = geometric mean titer

Thus, adjuvanted o-gp140 generated antigen-specific antibodies. Further, the antibodies were shown to increased in avidity over time.

30 C. Neutralizing Antibodies

Neutralizing antibodies post-third immunization were measured against HIV-1 SF2 in a T-cell line adapted virus

(TCLA) assay and against PBMC-grown HIV-1 variants SF2, SF162 and 119 using the CCR5+ CEMx174 LTR-GFP reporter cell line, 5.25 (provided by N. Landau, Salk Institute, San Diego, CA) as target cells. Results are shown in Table 13.

5

Table 13

Neutralizing antibody responses in rabbits immunized
with o-gp140.modUS4 protein

10

15

20

25

30

| Group | Animal | SF2 TCLA* | SF2 PBMC* | SF162 PBMC* | 119 PBMC* |
|--------------------------------------|--------|--------------|--------------|----------------|--------------|
| Experiment 1 | | | | | |
| o-gp140/ Ras-3c 50 mg | 217 | >640 | 100% | 49 | 17 |
| | 218 | >640 | 96 | 37 | 29 |
| Experiment 2 | | | | | |
| o-gp140/ MF59 50 mg | 792 | 45 | 71 | 39 | 26 |
| | 793 | 50 | 87 | 26 | 4 |
| | 794 | 59 | 87 | 13 | 0 |
| | 795 | 128 | 92 | 15 | 0 |
| o-gp140/ MF59 + MPL 50 mg | 804 | 173 | 91 | 47 | 18 |
| | 805 | 134 | 93 | 28 | 4 |
| | 806 | N.D.** | 95 | 49 | 13 |
| | 807 | 441 | 100 | 31 | 15 |
| o-gp140/MF59 + MPL + QHC 50 mg | 808 | 465 | 98 | 46 | 40 |
| | 809 | 496 | 100 | 44 | 39 |
| | 810 | >640 | 101 | 27 | 4 |
| | 811 | 92 | 92 | 24 | 37 |

*TCLA neutralizing antibody titers (50% inhibition).

**Not Determined

* % Inhibition at 1:10 dilution of sera with any detectable non-specific inhibition in pre-bleeds subtracted.

35

The above studies in rabbits indicate that the US4 o-gp140 protein is highly immunogenic. When administered with adjuvant, this protein was able to induce substantial antibody responses after only one or two immunizations.

5 Moreover, the adjuvanted o-gp140 protein was able to generate antigen-specific antibodies which increased in avidity after successive immunizations, and substantial neutralizing activity against T-cell line adapted HIV-1. Neutralizing activity was also observed against PBMC-grown
10 primary HIV strains, including the difficult to neutralize CCR5 co-receptor (R5)-utilizing isolates, SF162 and 119.

Example 10

In Vivo Immunogenicity of Synthetic Env Expression

Cassettes

A. General Immunization Methods

To evaluate the immunogenicity of the synthetic Env expression cassettes, studies using guinea pigs, rabbits, mice, rhesus macaques and baboons were performed. The
20 studies were structured as follows: DNA immunization alone (single or multiple); DNA immunization followed by protein immunization (boost); DNA immunization followed by Sindbis particle immunization; immunization by Sindbis particles alone.

B. Humoral Immune Response

The humoral immune response was checked in serum specimens from immunized animals with an anti-HIV Env antibody ELISAs (enzyme-linked immunosorbent assays) at various times post-immunization. The antibody titers of
30 the sera were determined by anti-Env antibody ELISA as described above. Briefly, sera from immunized animals were

screened for antibodies directed against the HIV gp120 or gp140 Env protein. Wells of ELISA microtiter plates were coated

overnight with the selected Env protein and washed four
5 times; subsequently, blocking was done with PBS-0.2% Tween
(Sigma) for 2 hours. After removal of the blocking
solution, 100 μ l of diluted mouse serum was added. Sera
were tested at 1/25 dilutions and by serial 3-fold
10 dilutions, thereafter. Microtiter plates were washed four
times and incubated with a secondary, peroxidase-coupled
anti-mouse IgG antibody (Pierce, Rockford, IL). ELISA
plates were washed and 100 μ l of 3, 3', 5, 5'-tetramethyl
benzidine (TMB; Pierce) was added per well. The optical
density of each well was measured after 15 minutes. Titers
15 are typically reported as the reciprocal of the dilution of
serum that gave a half-maximum optical density (O.D.).

Example 11

DNA-immunization of Baboons Using Synthetic Gag 20 Expression Cassettes

A. Baboons

Four baboons were immunized 3 times (weeks 0, 4 and 8)
bilaterally, intramuscular into the quadriceps using 1mg
pCMVKM2.GagMod.SF2 plasmid-DNA (Example 1). The animals
25 were bled two weeks after each immunization and a p24
antibody ELISA was performed with isolated plasma. The
ELISA was performed essentially as described in Example 5
except the second antibody-conjugate was an anti-human IgG,
g-chain specific, peroxidase conjugate (Sigma Chemical Co.,
30 St. Louis, MD 63178) used at a dilution of 1:500. Fifty
 μ g/ml yeast extract was added to the dilutions of plasma

samples and antibody conjugate to reduce non-specific background due to

preexisting yeast antibodies in the baboons. The antibody titer results are presented in Table 14.

5

Table 14

| Immunization no. | Weeks | Antigen | wpi */ Baboon No. | Ab-titer ^b |
|------------------|-------|--------------------|-------------------|-----------------------|
| 10 | 0 | gagmod DNA | 0 w/219 | < 10 |
| | | | 0 w/220 | < 10 |
| | | | 0 w/221 | < 10 |
| | | | 0 w/222 | < 10 |
| 15 | 6 | | 2 wp 1st/219 | < 10 |
| | | | 2 wp 1st/220 | < 10 |
| | | | 2 wp 1st/221 | < 10 |
| | | | 2 wp 1st/222 | 15 |
| 20 | 14 | gagmod DNA | 2 wp 4th/219 | < 10 |
| | | | 2 wp 4th/220 | 88 |
| | | | 2 wp 4th/221 | < 10 |
| | | | 2 wp 4th/222 | 56 |
| 25 | 30 | gagmod DNA | 2 wp 5th/219 | < 10 |
| | | | 2 wp 5th/220 | 391 |
| | | | 2 wp 5th/221 | 237 |
| | | | 2 wp 5th/222 | 222 |
| 30 | 46 | gag VLP protein | 2 wp 6th/219 | 753 |
| | | | 2 wp 6th/219 | 4330 |
| | | | 2 wp 6th/219 | 5000 |
| | | | 2 wp 6th/219 | 2881 |

^a wpi = weeks post immunization

^b geometric mean antibody titer

30

In Table 14, pre-bleed data are given as Immunization No. 0; data for bleeds taken 2 weeks post-first immunization are given as Immunization No. 1; data for bleeds taken 2 weeks post-second immunization are given as Immunization No. 2; and, data for bleeds taken 2 weeks post-third immunization are given as Immunization No. 3.

Further, lymphoproliferative responses to p24 antigen were also observed in baboons 221 and 222 two weeks post-fourth immunization (at week 14), and enhanced substantially post-boosting with VLP (at week 44 and 76).
5 Such proliferation results are indicative of induction of T-helper cell functions.

B. Rhesus Macaques

The improved potency of the codon-modified *gag* expression plasmid observed in mouse and baboon studies
10 was confirmed in rhesus macaques. Four of four macaques had detectable Gag-specific CTL after two or three 1 mg doses of modified *gag* plasmid. In contrast, in a previous study, only one of four macaques given 1 mg
15 doses of plasmid-DNA encoding the wild-type HIV-1_{SR2} Gag showed strong CTL activity that was not apparent until after the seventh immunization. Further evidence of the potency of the modified *gag* plasmid was the observation that CTL from two of the four rhesus macaques reacted
20 with three nonoverlapping Gag peptide pools, suggesting that as many as three different Gag peptides are recognized and indicating that the CTL response is polyclonal. Additional quantification and specificity studies are in progress to further characterize the T
25 cell responses to Gag in the plasmid-immunized rhesus macaques. DNA immunization of macaques with the modified *gag* plasmid did not result in significant antibody responses, with only two of four animals seroconverting at low titers. In contrast, in the same study the
30 majority of macaques in groups immunized with p55Gag protein seroconverted and had strong Gag-specific antibody titers. These data suggest that a prime-boost

strategy (DNA-prime and protein-boost) could be very promising for the induction of a strong CTL and antibody response.

In sum, these results demonstrate that the synthetic
5 Gag plasmid DNA is immunogenic in non-human primates. When similar experiments were carried out using wild-type Gag plasmid DNA no such induction of anti-p24 antibodies was observed after four immunizations.

10

Example 12

. DNA- and Protein Immunizations of Animals Using Env Expression Cassettes and Polypeptides

A. Guinea Pigs

Groups comprising six guinea pigs each were
15 immunized intramuscularly at 0, 4, and 12 weeks with plasmid DNAs encoding the gp120.modUS4, gp140.modUS4, gp140.modUS4.delV1, gp140.modUS4.delV2, gp140.modUS4.delV1/V2, or gp160.modUS4 coding sequences of the US4-derived Env. The animals were subsequently
20 boosted at 18 weeks with a single intramuscular dose of US4 o-gp140.mut.modUS4 protein in MF59 adjuvant. Anti-gp120 SF2 antibody titers (geometric mean titers) were measured at two weeks following the third DNA
immunization and at two weeks after the protein boost.
25 Results are shown in Table 15.

Table 15

| Group | GMT post-DNA immuniz. | GMT post-protein boost |
|---------------------------|--------------------------|---------------------------|
| gp120.modUS4 | 2098 | 9489 |
| gp140.modUS4 | 190 | 5340 |
| gp140.modUS4.delV1 | 341 | 7808 |
| gp140.modUS4.delV2 | 386 | 8165 |
| gp140.modUS4.delV1/V 2 | 664 | 8270 |
| gp160.modUS4 | 235 | 9928 |

These results demonstrate the usefulness of the synthetic constructs to generate immune responses, as well as, the advantage of providing a protein boost to enhance the immune response following DNA immunization.

B. Rabbits

Rabbits were immunized intramuscularly and intradermally using a Bioject needless syringe with plasmid DNAs encoding the following synthetic SF162 Env polypeptides: gp120.modSF162, gp120.modSF162.delV2, gp140.modSF162, gp140.modSF162.delV2, gp140.mut.modSF162, gp140.mut.modSF162.delV2, gp160.modSF162, and gp160.modSF162.delV2. Approximately 1 mg of plasmid DNA (pCMVlink) carrying the synthetic Env expression cassette was used to immunize the rabbits. Rabbits were immunized with plasmid DNA at 0, 4, and 12 weeks. At two weeks after the third immunization all of the constructs were shown to have generated significant antibody titers in the test animals. Further, rabbits immunized with constructs containing deletions of the V2 region

generally generated similar antibody titers relative to rabbits immunized with the companion construct still containing the V2 region.

The nucleic acid immunizations are followed by protein boosting with o-gp140.modSF162.delV2 (0.1 mg of purified protein) at 24 weeks after the initial immunization. Results are shown in Table 16.

Table 16

| Group | GMT 2wks post-2nd DNA immunization | GMT 2wks post-3rd DNA immunization | GMT 2wks post-protein boost |
|--------------------------|--|--|-----------------------------------|
| gp120.modSF162 | 4573 | 5899 | 26033 |
| gp120.modSF162.delV2 | 3811 | 3122 | 29606 |
| gp140.modSF162 | 1478 | 710 | 12882 |
| gp140.modSF162.delV2 | 1572 | 819 | 11067 |
| gp140.mut.modSF162 | 1417 | 788 | 8827 |
| gp140.mut.modSF162.delV2 | 1378 | 1207 | 13301 |
| gp160.modSF162 | 23 | 81 | 7050 |
| gp160.modSF162.delV2 | 85 | 459 | 11568 |

All constructs are highly immunogenic and generate substantial antigen binding antibody responses after only 2 immunizations in rabbits.

C. Baboons

Groups of four baboons were immunized intramuscularly with 1 mg doses of DNA encoding different forms of synthetic US4 gp140 (see the following table) at 0, 4, 8, 12, 28, and 44 weeks. The animals were also boosted twice with US4 0-gp140 protein (gp140.mut.modUS4) at 44 and 76 weeks using MF59 as adjuvant. Results are shown in Table 17.

| Table 17 | | | | |
|----------|------------------------------|--|---|---|
| Animal | Treatment | 2 Wks Post 5th DNA immuniza- tion | 2 Wks post 6th DNA (plus o- gp140 prot. immuniz.) | 2 Wks post 7th DNA (o-gp140 protein only) |
| CY 215 | gp140.modUS4 | 8.3 | 446 | 1813 |
| CY 216 | | 8.3 | 433 | 1236 |
| CY 217 | | 68 | 1660 | 2989 |
| CY 218 | | 101 | 2556 | 1610 |
| Geomean: | | 26.2 | 951.4 | 1812.1 |
| CY 219 | gp140.modUS4 + p55gag.SF2 | 8.3 | 8.3 | 421 |
| CY 220 | | 8.3 | 8.3 | 3117 |
| CY 221 | | 8.3 | 954 | 871 |
| CY 222 | | 8.3 | 71 | 916 |
| Geomean: | | 8.3 | 46.5 | 1011.5 |
| CY 223 | gp140.mut. modUS4 | 41.4 | 10497 | 46432 |
| CY 224 | | 8.3 | 979 | 470 |
| CY 225 | | 135 | 2935 | 3870 |
| CY 226 | | 47 | 1209 | 4009 |
| Geomean: | | 68.3 | 2457.4 | 4289.6 |
| CY 227 | gp140TM. modUS4 | 8.3 | 56 | 5001 |
| CY 228 | | 8.3 | 806 | 1170 |
| CY 229 | | 8.3 | 48 | 3402 |
| CY 230 | | 8.3 | 38 | 6520 |
| GMT*: | | 8.3 | 95.3 | 3375.3 |

*GMT = geometric mean titer

The results in Table 17 demonstrate the usefulness of the synthetic constructs to generate immune responses in primates such as baboons. In addition, all animals

showed evidence of antigen-specific (Env antigen) lymphoproliferative responses.

D. Rhesus Macaques

5 Two rhesus macaques (designated H445 and J408) were immunized with 1 mg of DNA encoding SF162 gp140 with a deleted V2 region (SF162.gp140.delV2) by intramuscular (IM) and intradermal (ID) routes at 0, 4, 8, and 28 weeks. Approximately 100 µg of the protein encoded by
10 the SF162. gp140mut.delV2 construct was also administered in MF59 by IM delivery at 28 weeks.

 ELISA titers are shown in Figure 61. Neutralizing antibody activity is shown Tables 18 and 19. Neutralizing antibody activity was determined against a
15 variety of primary HIV-1 isolates in a primary lymphocyte or "PBMC-based" assay (see the following tables). Further, the phenotypic co-receptor usage for each of the primary isolates is indicated. As can be seen in the tables neutralizing antibodies were detected against
20 every isolate tested, including the HIV-1 primary isolates (i.e., SF128A, 92US660, 92HT593, 92US657, 92US714, 91US056, and 91US054).

| Table 18 | | | | | |
|----------|------------------|------------------|-----------|-----------|----------------|
| Animal | Treatment | | Bleed 0 | Bleed 1 | Bleed 2 |
| | 1st Immunization | 2nd Immunization | 1st Imm'n | 2nd Imm'n | 2 Wks post 2nd |
| EO 456 | 25µg 120mod DNA | (None) | 8.3 | 45 | 309 |
| EO 457 | | | 8.3 | 254 | 460 |
| EO 458 | | | 8.3 | 8.3 | 93 |
| EO 459 | | | 8.3 | 43 | 45 |
| EO 460 | | | 8.3 | 8.3 | 274 |
| EO 461 | 25µg 120mod DNA | 25µg 120mod DNA | 8.3 | 47 | 1502 |
| EO 462 | | | 8.3 | 80 | 5776 |
| EO 463 | | | 8.3 | 89 | 3440 |
| EO 464 | | | 8.3 | 8.3 | 3347 |
| EO 465 | | | 8.3 | 69 | 1127 |
| EO 466 | 50µg 120mod DNA | (None) | 8.3 | 63 | 102 |
| EO 467 | | | 8.3 | 112 | 662 |
| EO 468 | | | 8.3 | 94 | 459 |
| EO 469 | | | 8.3 | 58 | 48 |
| EO 470 | | | 8.3 | 95 | 355 |
| EO 471 | 50µg 120mod DNA | 50µg 120mod DNA | 8.3 | 110 | 9074 |
| EO 472 | | | 8.3 | 8.3 | 4897 |
| EO 473 | | | 8.3 | 49 | 4089 |
| EO 474 | | | 8.3 | 59 | 5280 |
| EO 475 | | | 8.3 | 8.3 | 929 |
| EO 476 | 25µg 120mod DNA | Sindbis/Env | 8.3 | | 653 |
| EO 477 | | | 8.3 | 87 | 22675 |
| EO 478 | | | 8.3 | 76 | 3869 |
| EO 479 | | | 8.3 | | 1004 |
| EO 480 | | | 8.3 | 71 | 7080 |

| Table 19 | | | | | |
|----------|------------------|------------------|-----------|-----------|----------------|
| | Treatment | | Bleed 0 | Bleed 1 | Bleed 2 |
| Animal | 1st Immunization | 2nd Immunization | 1st Imm'n | 2nd Imm'n | 2 Wks post 2nd |
| EO 481 | Sindbis/Env | (None) | 8.3 | 8.3 | 8.3 |
| EO 482 | | | 8.3 | 8.3 | 8.3 |
| EO 483 | | | 8.3 | 78 | 103 |
| EO 484 | | | 8.3 | 8.3 | 32 |
| EO 485 | | | 8.3 | 76 | 207 |
| EO 486 | Sindbis/Env | Sindbis/Env | 8.3 | 8.3 | 458 |
| EO 487 | | | 8.3 | 8.3 | 345 |
| EO 488 | | | 8.3 | 8.3 | 331 |
| EO 489 | | | 8.3 | 103 | 111 |
| EO 490 | | | 8.3 | 8.3 | 5636 |

Lymphoproliferative activity (LPA) was also determined by antigenic stimulation followed by uptake of ³H-thymidine in these animals and is shown in Table 20.

Experiment 1 was performed at 14 weeks post third DNA immunization and Experiment 2 was performed at 2 weeks post fourth DNA immunization using DNA and protein. For gp120ThaiE, gp120SF2 and US4 o-gp140, appropriate background values were used to calculate Stimulation Indices (S.I.; Antigenic stimulation CPM/Background CPM).

Table 20

| S.I.: Calculated as Ag CPM/Background CPM | | | | |
|---|----------------|-----------|-----------|----------------|
| Animal/ exp# | gp120Thai E | gp120 SF2 | env2-3SF2 | o- gp140US4 |
| J408/#1 | 2 | 1 | 1 | 5 |
| H445/#1 | 1 | 1 | 1 | 6 |
| J408/#2 | 1 | 1 | 2 | 3 |
| H445/#2 | 0 | 0 | 3 | 2 |

As can be seen by the results presented in Table 20 lymphoproliferative responses to o-gp140.US4 antigen were also in all four animals at both experimental time points. Such proliferation results are indicative of induction of T-helper cell functions.

The results presented above demonstrate that the synthetic gp140.modSF162.delV2 DNA and protein are immunogenic in non-human primates.

Example 13

In vitro expression of recombinant Sindbis RNA and
DNA containing the synthetic Gag or Env expression
cassettes

5 A. Synthetic Gag expression cassettes

To evaluate the expression efficiency of the
synthetic Gag expression cassette in Alphavirus vectors,
the synthetic Gag expression cassette was subcloned into
both plasmid DNA-based and recombinant vector particle-
10 based Sindbis virus vectors. Specifically, a cDNA vector
construct for *in vitro* transcription of Sindbis virus RNA
vector replicons (pRSIN-luc; Dubensky, et al., *J Virol.*
70:508-519, 1996) was modified to contain a *PmeI* site for
plasmid linearization and a polylinker for insertion of
15 heterologous genes. A polylinker was generated using two
oligonucleotides that contain the sites *XhoI*, *PmlI*, *ApaI*,
NarI, *XbaI*, and *NotI* (XPANXNF, SEQ ID NO:17, and XPANXNR,
SEQ ID NO:18).

The plasmid pRSIN-luc (Dubensky et al., *supra*) was
20 digested with *XhoI* and *NotI* to remove the luciferase gene
insert, blunt-ended using Klenow and dNTPs, and purified
from an agarose gel using GeneCleanII (Biol01, Vista,
CA). The oligonucleotides were annealed to each other
and ligated into the plasmid. The resulting construct
25 was digested with *NotI* and *SacI* to remove the minimal
Sindbis 3'-end sequence and A₄₀ tract, and ligated with an
approximately 0.4 kbp fragment from PKSSIN1-BV (WO
97/38087). This 0.4 kbp fragment was obtained by
digestion of pKSSIN1-BV with *NotI* and *SacI*, and
30 purification after size fractionation from an agarose
gel. The fragment contained the complete Sindbis virus
3'-end, an A₄₀ tract and a *PmeI* site for linearization.
This new vector construct was designated SINBVE.

The synthetic HIV Gag coding sequence was obtained from the parental plasmid by digestion with *EcoRI*, blunt-ending with Klenow and dNTPs, purification with GeneCleanII, digestion with *SalI*, size fractionation on an agarose gel, and purification from the agarose gel using GeneCleanII. The synthetic Gag coding fragment was ligated into the SINBVE vector that had been digested with *XhoI* and *PmlI*. The resulting vector was purified using GeneCleanII and designated SINBVGag. Vector RNA replicons may be transcribed *in vitro* (Dubensky et al., *supra*) from SINBVGag and used directly for transfection of cells. Alternatively, the replicons may be packaged into recombinant vector particles by co-transfection with defective helper RNAs or using an alphavirus packaging cell line as described, for example, in U.S. Patent Numbers 5,843,723 and 5,789,245, and then administered *in vivo* as described..

The DNA-based Sindbis virus vector pDCMVSIN-beta-gal (Dubensky, et al., *J Virol.* 70:508-519, 1996) was digested with *SalI* and *XbaI*, to remove the beta-galactosidase gene insert, and purified using GeneCleanII after agarose gel size fractionation. The HIV Gag gene was inserted into the the pDCMVSIN-beta-gal by digestion of SINBVGag with *SalI* and *XhoI*, purification using GeneCleanII of the Gag-containing fragment after agarose gel size fractionation, and ligation. The resulting construct was designated pDSIN-Gag, and may be used directly for *in vivo* administration or formulated using any of the methods described herein.

BHK and 293 cells were transfected with recombinant Sindbis vector RNA and DNA, respectively. The supernatants and cell lysates were tested with the Coulter p24 capture ELISA (Example 2).

BHK cells were transfected by electroporation with recombinant Sindbis RNA. The expression of p24 (in ng/ml) is presented in Table 21. In the table, SINGag#1 and 2 represent duplicate measurements, and SIN β gal represents a negative control. Supernatants and lysates were collected 24h post transfection.

Table 21

| Construct | Supernatant | Lysate |
|---------------------|-------------|-------------------------|
| SIN β gal RNA | 0 | 0 |
| SINGag#1 RNA | 7 ng | Max (approx. 1 μ g) |
| SINGag#2 RNA | 1 ng | 700 ng |

293 cells were transfected using LT-1 (Example 2) with recombinant Sindbis DNA. Synthetic pCMVKM2GagMod.SF2 was used as a positive control. Supernatants and lysates were collected 48h post transfection. The expression of p24 (in ng/ml) is presented in Table 22.

Table 22

| Construct | Supernatant | Lysate |
|------------------------|-------------|--------|
| SINGag DNA | 3 | 30 |
| pCMVKM2.GagMod.SF2 DNA | 32 | 42 |

The results presented in Tables 21 and 22 demonstrate that Gag proteins can be efficiently expressed from both DNA and RNA-based Sindbis vector systems using the synthetic Gag expression cassette (p55Gag.mod).

B. Synthetic Env expression cassettes

To evaluate the expression efficiency of the synthetic Env expression cassette in Alphavirus vectors,

synthetic Env expression cassettes were subcloned into both plasmid DNA-based and recombinant vector particle-based Sindbis virus vectors as described above for Gag.

The synthetic HIV Env coding sequence was obtained from the parental plasmid by digestion with *SalI* and *XbaI*, size fractionation on an agarose gel, and purification from the agarose gel using GeneCleanII. The synthetic Env coding fragment was ligated into the SINBVE vector that had been digested with *XhoI* and *XbaI*. The resulting vector was purified using GeneCleanII and designated SINBVEEnv. Vector RNA replicons may be transcribed *in vitro* (Dubensky et al., *supra*) from SINBVEEnv and used directly for transfection of cells. Alternatively, the replicons may be packaged into recombinant vector particles by co-transfection with defective helper RNAs or using an alphavirus packaging cell line and administered as described above for Gag.

The DNA-based Sindbis virus vector pDCMVSIN-beta-gal (Dubensky, et al., *J Virol.* 70:508-519, 1996) was digested with *SalI* and *XbaI*, to remove the beta-galactosidase gene insert, and purified using GeneCleanII after agarose gel size fractionation. The HIV Env gene was inserted into the the pDCMVSIN-beta-gal by digestion of SINBVEEnv with *XbaI* and *XhoI*, purification using GeneCleanII of the Env-containing fragment after agarose gel size fractionation, and ligation. The resulting construct was designated pDSIN-Env, and may be used directly for *in vivo* administration or formulated using any of the methods described herein.

BHK and 293 cells were transfected with recombinant Sindbis vector RNA and DNA, respectively. The supernatants and cell lysates were tested by capture ELISA.

BHK cells were transfected by electroporation with recombinant Sindbis RNA. The expression of Env (in ng/ml) is presented in Table 23. In the table, the Sindbis RNA containing synthetic Env expression cassettes are indicated and β gal represents a negative control. Supernatants and lysates were collected 24h post transfection.

Table 23

| Construct | Supernatant (Neat)ng/ml | Lysate (1:10 dilution)ng/ml |
|-----------------------|----------------------------|--------------------------------|
| β gal RNA | 0 | 0 |
| gp140.modUS4 | 726 | 7147 |
| gp140.modSF162 | 3529 | 7772 |
| gp140.modUS4.delV1/V2 | 1738 | 6526 |
| gp140.modUS4.delV2 | 960 | 3023 |
| gp140.modSF162.delV2 | 2772 | 3359 |

293 cells were transfected using LT-1 mediated transfection (PanVera) with recombinant Sindbis DNA containing synthetic expression cassettes of the present invention and β gal sequences as a negative control. Supernatants and lysates were collected 48h post transfection. The expression of Env (in ng/ml) is presented in Table 24.

Table 24

| Construct | Supernatant (Neat) ng/ml | Lysate (1:10 dilution) ng/ml |
|----------------------|-----------------------------|------------------------------------|
| β gal | 0 | 0 |
| gp140.modSF162.delV2 | 1977 | 801 |
| gp140.modSF162 | 949 | 746 |

The results presented in Tables 23 and 24 demonstrated that Env proteins can be efficiently expressed from both DNA and RNA-based Sindbis vector systems using the synthetic Env expression cassettes of the present invention.

Example 14

A. In vivo Immunization with Gag-containing DNA and/or Sindbis particles

CB6F1 mice were immunized intramuscularly at 0 and 4 weeks with plasmid DNA and/or Sindbis vector RNA-containing particles each containing GagMod.SF2 sequences as indicated in Table 25. Animals were challenged with recombinant vaccinia expressing SF2 Gag at 3 weeks post second immunization (at week 7). Spleens were removed from the immunized and challenged animals 5 days later for a standard ^{51}C release assay for CTL activity. Values shown in Table 25 indicate the results from the spleens of three mice from each group. The boxed values in Table 25 indicate that all groups of mice receiving immunizations with pCMVKm2.GagMod.SF2 DNA and/or SindbisGagMod.SF2 virus particles either alone or in combinations showed antigen-specific CTL activity.

Table 25

Cytotoxic T-lymphocyte (CTL) responses in mice immunized with HIV-1 gagmod DNA and Sindbis gagmod virus particles

| Immunization | E:T | Percent specific lysis of target cells* | | |
|--|-------|---|------------|---------|
| | | SVBALB none | SVBALB p7g | RMA p7g |
| pCMVKm2.GagMod.SF2 DNA ^a | 100:1 | 5 | 20 | 1 |
| at 0, 4 wks | 25:1 | 5 | 20 | <1 |
| | 6:1 | 4 | 8 | <1 |
| SindbisGagMod.SF2 virus particles ^b | 100:1 | 10 | 49 | <1 |
| at 0, 4 weeks | 25:1 | 7 | 20 | <1 |
| | 6:1 | 5 | 12 | <1 |
| pCMVKm2.GagMod.SF2 DNA at 0 wks | 100:1 | 9 | 58 | <1 |
| SindbisGagMod.SF2 virus particles at 4 wks | 25:1 | 7 | 42 | 2 |
| | 6:1 | 4 | 13 | <1 |
| SindbisGagMod.SF2 virus particles at 4 wks | 100:1 | 5 | 38 | <1 |
| | 25:1 | 4 | 18 | <1 |
| pCMVKm2.GagMod.SF2 DNA at 0 wks | 6:1 | 3 | 13 | 1 |

^a 20 µg

^b 10⁷ particles

* Challenge with recombinant vaccinia virus expressing HIV-1SF2 Gag at 3 weeks post second immunization (week 7). Spleens taken 5 days later. Ex vivo CTL assay performed by standard ⁵¹Cr release assay. Values seen represent results from 3 pooled mouse spleens per group

B. In vivo Immunization with Env-containing DNA and/or Sindbis particles

Balb/C mice were immunized intramuscularly at 0 and 4 weeks(as shown in the following table) with plasmid DNA and/or Sindbis-virus RNA-containing particles each containing gp120.modUS4 sequences. Treatment regimes and antibody titers are shown in Table 26. Antibody titers were determined by ELISA using gp120 SF2 protein to coat the plates.

| Table 26 | | | | | |
|----------|-----------------------|-----------------------|-----------|-----------------|------------------|
| | Treatment | | Bleed 0 | Bleed 1 (8 wks) | Bleed 2 (10 wks) |
| Animal | 1st Immunization | 2nd Immunization | 1st Imm'n | 2nd Imm'n | 2 Wks post 2nd |
| EO 456 | 25 μ g 120mod DNA | (None) | 8.3 | 45 | 309 |
| EO 457 | | | 8.3 | 254 | 460 |
| EO 458 | | | 8.3 | 8.3 | 93 |
| EO 459 | | | 8.3 | 43 | 45 |
| EO 460 | | | 8.3 | 8.3 | 274 |
| EO 461 | 25 μ g 120mod DNA | 25 μ g 120mod DNA | 8.3 | 47 | 1502 |
| EO 462 | | | 8.3 | 80 | 5776 |
| EO 463 | | | 8.3 | 89 | 3440 |
| EO 464 | | | 8.3 | 8.3 | 3347 |
| EO 465 | | | 8.3 | 69 | 1127 |
| EO 466 | 50 μ g 120mod DNA | (None) | 8.3 | 63 | 102 |
| EO 467 | | | 8.3 | 112 | 662 |
| EO 468 | | | 8.3 | 94 | 459 |
| EO 469 | | | 8.3 | 58 | 48 |
| EO 470 | | | 8.3 | 95 | 355 |
| EO 471 | 50 μ g 120mod DNA | 50 μ g 120mod DNA | 8.3 | 110 | 9074 |
| EO 472 | | | 8.3 | 8.3 | 4897 |
| EO 473 | | | 8.3 | 49 | 4089 |
| EO 474 | | | 8.3 | 59 | 5280 |
| EO 475 | | | 8.3 | 8.3 | 929 |
| EO 476 | 25 μ g 120mod DNA | Sindbis/Env | 8.3 | | 653 |
| EO 477 | | | 8.3 | 87 | 22675 |
| EO 478 | | | 8.3 | 76 | 3869 |
| EO 479 | | | 8.3 | | 1004 |
| EO 480 | | | 8.3 | 71 | 7080 |
| EO 481 | Sindbis/Env | (None) | 8.3 | 8.3 | 8.3 |
| EO 482 | | | 8.3 | 8.3 | 8.3 |
| EO 483 | | | 8.3 | 78 | 103 |
| EO 484 | | | 8.3 | 8.3 | 32 |
| EO 485 | | | 8.3 | 76 | 207 |
| EO 486 | Sindbis/Env | Sindbis/Env | 8.3 | 8.3 | 458 |
| EO 487 | | | 8.3 | 8.3 | 345 |
| EO 488 | | | 8.3 | 8.3 | 331 |
| EO 489 | | | 8.3 | 103 | 111 |
| EO 490 | | | 8.3 | 8.3 | 5636 |

As can be seen from the data presented above, all of the mice generally demonstrated substantial immunological responses by bleed number 2. For Env, the best results were obtained using either (i) 50 μ g of gp120.modUS4 DNA for the first immunization followed by a second

immunization using 50 μ g of gp120.modUS4 DNA, or (ii) 25 μ g of gp120.modUS4 DNA for the first immunization followed by a second immunization using 10^7 pfus of Sindbis.

5 The results presented above demonstrate that the Env and Gag proteins of the present invention are effective to induce an immune response using Sindbis vector systems which include the synthetic Env (e.g., gp120.modUS4) or Gag expression cassettes.

10

Example 15

Co-Transfection of Env and Gag as Monocistronic and Bicistronic Constructs

15 DNA constructs encoding (i) wild-type US4 and SF162 Env polypeptides, (ii) synthetic US4 and SF162 Env polypeptides (gp160.modUS4, gp160.modUS4.delV1/V2, gp160.modSF162, and gp120.modSF162.delV2), and (iii) SF2gag polypeptide (i.e., the Gag coding sequences obtained from the SF2 variant or optimized sequences
20 corresponding to the gagSF2 -- gag.modSF2) were prepared. These monocistronic constructs were co-transfected into 293T cells in a transient transfection protocol using the following combinations: gp160.modUS4; gp160.modUS4 and gag.modSF2; gp160.modUS4.delV1/V2; gp160.modUS4.delV1/V2
25 and gag.modSF2; gp160.modSF162 and gag.modSF2; gp120.modSF162.delV2 and gag.modSF2; and gag.modSF2 alone.

30 Further several bicistronic constructs were made where the coding sequences for Env and Gag were under the control of a single CMV promoter and, between the two coding sequences, an IRES (internal ribosome entry site (EMCV IRES); Kozak, M., Critical Reviews in Biochemistry and Molecular Biology 27(45):385-402, 1992; Witherell, G.W., et al., Virology 214:660-663, 1995) sequence was

introduced after the Env coding sequence and before the
Gag coding sequence. Those constructs were as follows:
gp160.modUS4.gag.modSF2, SEQ ID NO:73 (Figure 61);
gp160.modUSF162.gag.modSF2, SEQ ID NO:74 (Figure 62);
5 gp160.modUS4.delV1/V2.gag.modSF2, SEQ ID NO:75 (Figure
63); and gp160.modSF162.delV2.gag.modSF2, SEQ ID NO:76
(Figure 64).

Supernatants from cell culture were filtered through
0.45 μ m filters then ultracentrifuged for 2 hours at
10 24,000 rpm (140,000Xg) in an SW28 rotor through a 20%
sucrose cushion. The pelleted materials were suspended
and layered on a 20-60% sucrose gradient and spun for 2
hours at 40,000 rpm (285,000Xg) in an SW41Ti rotor.
Gradients were fractionated into 1.0 ml samples. A total
15 of 9-10 fractions were typically collected from each DNA
transfection group.

The fractions were tested for the presence of the
Env and Gag proteins (across all fractions). These
results demonstrated that the appropriate proteins were
20 expressed in the transfected cells (i.e., if an Env
coding sequence was present the corresponding Env protein
was detected; if a Gag coding sequence was present the
corresponding Gag protein was detected).

Virus like particles (VLPs) were known to be present
25 through a selected range of sucrose densities. Chimeric
virus like particles (VLPs) were formed using all the
tested combinations of constructs containing both Env and
Gag. Significantly more protein was found in the
supernatant collected from the cells transfected with
30 "gp160.modUS4.delV1/V2 and gag.modSF2" than in all the
other supernatants.

Western blot analysis was also performed on sucrose
gradient fractions from each transfection. The results
show that bicistronic plasmids gave lower amounts of VLPs

than the amounts obtained using co-transfection with monocistronic plasmids.

In order to verify the production of chimeric VLPs by these cell lines the following electron microscopic analysis was carried out.

293T cells were plated at a density of 60-70% confluence in 100 mm dishes on the day before transfection. The cells were transfected with 10 µg of DNA in transfection reagent LT1 (Panvera Corporation, 545 Science Dr., Madison, WI). The cells were incubated overnight in reduced serum medium (opti-MEM, Gibco-BRL, Gaithersburg, MD). The medium was replaced with 10% fetal calf serum, 2% glutamine in IMDM in the morning of the next day and the cells were incubated for 65 hours. Supernatants and lysates were collected for analysis as described above (see Example 2).

The fixed, transfected 293T cells and purified ENV-GAG VLPs were analyzed by electron microscopy. The cells were fixed as follows. Cell monolayers were washed twice with PBS and fixed with 2% glutaraldehyde. For purified VLPs, gradient peak fractions were collected and concentrated by ultracentrifugation (24,000 rpm) for 2 hours. Electron microscopic analysis was performed by Prof. T.S. Benedict Yen (Veterans Affairs, Medical Center, San Francisco, CA).

Electron microscopy was carried out using a transmission electron microscope (Zeiss 10c). The cells were pre-stained with osmium and stained with uranium acetate and lead citrate. Immunostaining was performed to visualize envelope on the VLP. The magnification was 100,000X.

Figures 65A-65F show micrographs of 293T cells transfected with the following constructs: Figure 65A, gag.modSF2; Figure 65B, gp160.modUS4; Figure 65C,

gp160.modUS4.delV1/V2.gag.modSF2 (bicistronic Env and
Gag); Figures 65D and 65E, gp160.modUS4.delV1/V2 and
gag.modSF2; and Figure 65F, gp120.modSF162.delV2 and
gag.modSF2. In the figures, free and budding immature
5 virus-like-particles (VLPs) of the expected size
(approximately 100 nm) decorated with the Env protein
were seen. In sum, gp160 polypeptides incorporate into
Gag VLPs when constructs were co-transfected into cells.
The efficiency of incorporation is 2-3 fold higher when
10 constructs encoding V-deleted Env polypeptides from high
synthetic expression cassettes are used.

Although preferred embodiments of the subject
invention have been described in some detail, it is
understood that obvious variations can be made without
15 departing from the spirit and the scope of the invention
as defined by the appended claims.

What Is Claimed Is:

1. An expression cassette, comprising
5 a polynucleotide sequence encoding a polypeptide including an HIV Gag polypeptide, wherein the polynucleotide sequence encoding said Gag polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:20.
10
2. The expression cassette of claim 1, comprising,
a polynucleotide sequence encoding a polypeptide including an HIV Gag polypeptide, wherein the
polynucleotide sequence encoding said Gag polypeptide
15 comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:9.
3. The expression cassette of claim 1, wherein said
polynucleotide sequence encoding a polypeptide including
20 an HIV Gag polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:4.
4. The expression cassette of claim 1, wherein said
25 polynucleotide sequence further includes a polynucleotide sequence encoding an HIV protease polypeptide.
5. The expression cassette of claim 4, wherein the
nucleotide sequence encoding said polypeptide comprises a
30 sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:78, and SEQ ID NO:79.
6. The expression cassette of claim 1, wherein said

polynucleotide sequence further includes a polynucleotide sequence encoding an HIV reverse transcriptase polypeptide.

5 7. The expression cassette of claim 6, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, and SEQ
10 ID NO:84.

 8. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV tat polypeptide.

15 9. The expression cassette of claim 8, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88 and SEQ ID NO:89.

 10. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV polymerase
25 polypeptide, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:6.

30 11. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV polymerase polypeptide, wherein (i) the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90%

sequence identity to the sequence presented as SEQ ID NO:4, and (ii) wherein the sequence is modified by deletions of coding regions corresponding to reverse transcriptase and integrase.

5

12. The expression cassette of claim 11, wherein said polynucleotide sequence preserves T-helper cell and CTL epitopes.

10

13. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HCV core polypeptide, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:7.

15

14. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:71 (Figure 58) or SEQ ID NO:72 (Figure 59).

20

15. The expression cassette of claim 14, wherein said *Env* polypeptide includes sequences flanking a V1 region but has a deletion in the V1 region itself.

25

16. The expression cassette of claim 15, wherein the polynucleotide sequence encoding the polypeptide comprises the sequence presented as SEQ ID NO:65 (Figure 52 gp160.modUS4.delV1).

30

17. The expression cassette of claim 14, wherein

said Env polypeptide includes sequences flanking a V2 region but has a deletion in the V2 region itself.

18. The expression cassette of claim 17, wherein
5 the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:60 (Figure 47); and SEQ ID NO:66 (Figure 53).

19. The expression cassette of claim 17, wherein
10 the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:34 (Figure 20); SEQ ID NO:37 (Figure 24); SEQ ID NO:40 (Figure 27); SEQ ID NO:43 (Figure 30); SEQ ID NO:46 (Figure 33); SEQ ID NO:49 (Figure 36); and SEQ ID NO:76
15 (Figure 64).

20. The expression cassette of claim 14, wherein
said Env polypeptide includes sequences flanking a V1/V2 region but has a deletion in the V1/V2 region itself.
20

21. The expression cassette of claim 20, wherein
the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:59 (Figure 46); SEQ ID NO:61 (Figure 48); SEQ ID NO:67
25 (Figure 54); and SEQ ID NO:75 (Figure 63).

22. The expression cassette of claim 20, wherein
the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:35
30 (Figure 21); SEQ ID NO:38 (Figure 25); SEQ ID NO:41 (Figure 28); SEQ ID NO:44 (Figure 31); SEQ ID NO:47 (Figure 34) and SEQ ID NO:50 (Figure 37).

23. The expression cassette of claim 14, wherein said Env polypeptide has a mutated cleavage site that prevents the cleavage of a gp140 polypeptide into a gp120 polypeptide and a gp41 polypeptide.

5

24. The expression cassette of claim 23, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:57 (Figure 44); SEQ ID NO:61 (Figure 48); and SEQ ID NO:63 (Figure 50).

10

25. The expression cassette of claim 23, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:39 (Figure 26); SEQ ID NO:40 (Figure 27); SEQ ID NO:41 (Figure 28); SEQ ID NO:42 (Figure 29); SEQ ID NO:43 (Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45 (Figure 32); SEQ ID NO:46 (Figure 33); and SEQ ID NO:47 (Figure 34).

15

20

26. The expression cassette of claim 14, wherein said Env polypeptide includes a gp160 Env polypeptide or a polypeptide derived from a gp160 Env polypeptide.

25

27. The expression cassette of claim 26, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:64 (Figure 51); SEQ ID NO:65 (Figure 52); SEQ ID NO:66 (Figure 53); SEQ ID NO:67 (Figure 54); SEQ ID NO:68 (Figure 55); SEQ ID NO:75 (Figure 63); and SEQ ID NO:73 (Figure 61).

30

28. The expression cassette of claim 26, wherein

the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:48 (Figure 35); SEQ ID NO:49 (Figure 36); SEQ ID NO:50 (Figure 37); SEQ ID NO:76 (Figure 64); and SEQ ID NO:74 (Figure 62).

29. The expression cassette of claim 14, wherein said *Env* polypeptide includes a gp140 *Env* polypeptide or a polypeptide derived from a gp140 *Env* polypeptide.

30. The expression cassette of claim 29, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:56 (Figure 43); SEQ ID NO:57 (Figure 44); SEQ ID NO:58 (Figure 45); SEQ ID NO:59 (Figure 46); SEQ ID NO:60 (Figure 47); SEQ ID NO:61 (Figure 48); SEQ ID NO:62 (Figure 49); and SEQ ID NO:63 (Figure 50).

31. The expression cassette of claim 29, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:36 (Figure 23); SEQ ID NO:37 (Figure 24); SEQ ID NO:38 (Figure 25); SEQ ID NO:39 (Figure 26); SEQ ID NO:40 (Figure 27); SEQ ID NO:41 (Figure 28); SEQ ID NO:42 (Figure 29); SEQ ID NO:43 (Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45 (Figure 32); SEQ ID NO:46 (Figure 33); and SEQ ID NO:47 (Figure 34).

32. The expression cassette of claim 14, wherein said *Env* polypeptide includes a gp120 *Env* polypeptide or a polypeptide derived from a gp120 *Env* polypeptide.

33. The expression cassette of claim 32, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:54 (Figure 41); and SEQ ID NO:55 (Figure 42).

5

34. The expression cassette of claim 32, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:33 (Figure 19); SEQ ID NO:34 (Figure 20); and SEQ ID NO:35 (Figure 21).

10

35. The expression cassette of claim 14, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:55 (Figure 42); SEQ ID NO:62 (Figure 49); SEQ ID NO:63 (Figure 50); and SEQ ID NO:68 (Figure 55).

15

36. A recombinant expression system for use in a selected host cell, comprising, an expression cassette of any of claims 1-35, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected host cell.

20

37. The recombinant expression system of claim 36, wherein said control elements are selected from the group consisting of a transcription promoter, a transcription enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences.

25

30

38. The recombinant expression system of claim 36, wherein said transcription promoter is selected from the

group consisting of CMV, CMV+intron A, SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein.

5 39. A cell comprising an expression cassette of any of claims 1-35, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected cell.

10 40. The cell of claim 39, wherein the cell is a mammalian cell.

15 41. The cell of claim 40, wherein the cell is selected from the group consisting of BHK, VERO, HT1080, 293, RD, COS-7, and CHO cells.

42. The cell of claim 41, wherein said cell is a CHO cell.

20 43. The cell of claim 39, wherein the cell is an insect cell.

44. The cell of claim 43, wherein the cell is either *Trichoplusia ni* (Tn5) or Sf9 insect cells.

25 45. The cell of claim 39, wherein the cell is a bacterial cell.

30 46. The cell of claim 39, wherein the cell is a yeast cell.

47. The cell of claim 39, wherein the cell is a plant cell.

48. The cell of claim 39, wherein the cell is an antigen presenting cell.

49. The cell of claim 48, wherein the lymphoid cell
5 is selected from the group consisting of macrophage, monocytes, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof.

50. The cell of claim 39, wherein the cell is a
10 primary cell.

51. The cell of claim 39, wherein the cell is an immortalized cell.

52. The cell of claim 39, wherein the cell is a
15 tumor-derived cell.

53. A method for producing a polypeptide including HIV Gag polypeptide sequences, said method comprising,
20 incubating the cells of claim 39, under conditions for producing said polypeptide.

54. A method for producing virus-like particles (VLPs), comprising,
25 incubating the cells of claim 39, under conditions for producing said VLPs.

55. A method for producing a composition of virus-like particles (VLPs), comprising,
30 (a) incubating the cells of claim 39, under conditions for producing said VLPs; and
(b) substantially purifying said VLPs to produce a composition of VLPs.

56. A cell line useful for packaging lentivirus vectors, comprising

5 suitable host cells that have been transfected with an expression vector containing an expression cassette of any of claims 1-35, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell.

10 57. The cell line of claim 56, wherein suitable host cells have been transfected with an expression vector containing the expression cassette of any of claims 1-13.

15 58. The cell line of claim 56, wherein suitable host cells have been transfected with an expression vector containing the expression cassette of claim 1-3.

20 59. The cell line of claim 56, wherein suitable host cells have been transfected with an expression vector containing the expression cassette of claim 14-35.

60. A gene delivery vector for use in a Mammalian subject, comprising

25 a suitable gene delivery vector for use in said subject, wherein the vector comprises an expression cassette of any of claims 1-35, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the subject.

30 61. A method of DNA immunization of a subject, comprising,

introducing a gene delivery vector of claim 60 into said subject under conditions that are compatible with expression of said expression cassette in said subject.

62. The method of claim 61, wherein said gene delivery vector is a nonviral vector.

5 63. The method of claim 61, wherein said vector is delivered using a particulate carrier.

64. The method of claim 63, wherein said vector is coated on a gold or tungsten particle and said coated
10 particle is delivered to said subject using a gene gun.

65. The method of claim 63, wherein said vector is encapsulated in a liposome preparation.

15 66. The method of claim 61, wherein said vector is a viral vector.

67. The method of claim 66, wherein said viral vector is a retroviral vector.

20 68. The method of claim 67, wherein said viral vector is a lentiviral vector.

69. The method of claim 61, wherein said subject is
25 a mammal.

70. The method of claim 69, wherein said mammal is a human.

30 71. A method of generating an immune response in a subject, comprising
transfecting cells of said subject a gene delivery vector of claim 60, under conditions that permit the expression of said polynucleotide and production of said

polypeptide, thereby eliciting an immunological response to said polypeptide.

5 72. The method of claim 71, wherein said vector is a nonviral vector.

73. The method of claim 72, wherein said vector is delivered using a particulate carrier.

10 74. The method of claim 73, wherein said vector is coated on a gold or tungsten particle and said coated particle is delivered to said vertebrate cell using a gene gun.

15 75. The method of claim 73, wherein said vector is encapsulated in a liposome preparation.

20 76. The method of claim 71, wherein said vector is a viral vector.

77. The method of claim 76, wherein said viral vector is a retroviral vector.

25 78. The method of claim 77, wherein said viral vector is a lentiviral vector.

79. The method of claim 71, wherein said subject is a mammal.

30 80. The method of claim 79, wherein said mammal is a human.

81. The method of claim 71, wherein said transfecting is done ex vivo and said transfected cells

are reintroduced into said subject.

82. The method of claim 71, wherein said transfecting is done *in vivo* in said subject.

5

83. The method of claim 71, where said immune response is a humoral immune response.

84. The method of claim 71, where said immune response is a cellular immune response.

10

85. A gene delivery vector comprising an alphavirus vector construct, wherein said alphavirus construct comprises an expression cassette according to any one of claims 1 through 35.

15

86. The gene delivery vector of claim 85, wherein the alphavirus vector construct is a cDNA vector construct.

20

87. The gene delivery vector of claim 85, wherein the alphavirus comprises a recombinant alphavirus particle preparation.

88. The gene delivery vector of claim 85, wherein the vector comprises a eukaryotic layered vector initiation system.

25

89. A method of stimulating an immune response in a subject comprising administering the gene delivery vector of any one of claims 85 through 88 in an amount effective to stimulate an immune response in said subject.

30

90. The method of claim 89, wherein the gene

delivery vector is administered intramuscularly, intramucosally, intranasally, subcutaneously, intradermally, transdermally, intravaginally, intrarectally, orally or intravenously.

5

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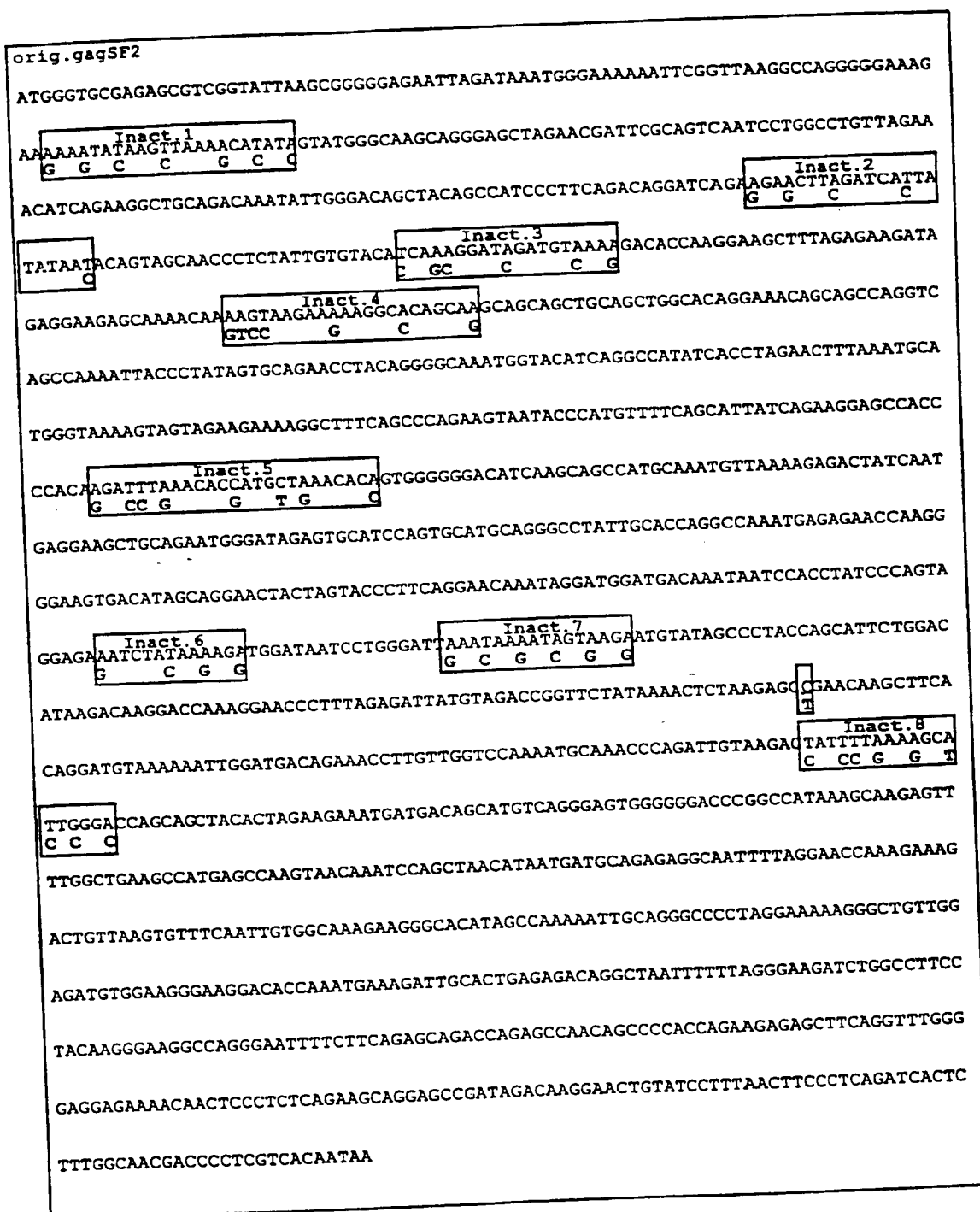


FIG. 1

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From here codon optimization + inactivation (GP1) and (GP2)

ATGGGTGCGAGAGCGTCGGTATTAAGCGGGGAGAAATTAGATAAAATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAG

Inact. 1
AAAAATATAAGTTAAACATATGATATGGGCAAGCAGGGAGCTAGAACGATTGCGAGTCAATCCTGGCCTGTTAGAA
G G C C G C C

ACATCAGAAGGCTGCAGACAAATATTGGGACAGCTACAGCCATCCCTTCAGACAGGATCAGAGAACTTAGATCATTAA
Inact. 2
G G C C C

Inact. 2
TATAATACAGTAGCAACCCCTCTATTGTGTACATCAAGGATAGATGTAAAGACACCAAGGAAGCTTTAGAGAAGATA
C C C C C C C

Inact. 4
GAGGAAGAGCAAAACAAAGTAAGAAAAAGGCACAGCAAGCAGCAGCTGCAGCTGGCACAGGAAACAGCAGCCAGGTC
G T C C G C C

AGCCAAAATTACCTTATAGTGCAGAACCTACAGGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCA

TGGGTAAAAGTAGTAGAAGAAAAGGCTTTTTCAGCCCAGAAGTAATACCCATGTTTTTCAGCATTATCAGAAGGAGCCACC

Inact. 5
CCACAGATTTTAAACACCATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAATGTTAAAAGAGACTATCAAT
G C C G G T G C

GAGGAAGCTGCAGAATGGGATAGAGTGCATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAAATGAGAGAACCAAGG

GGAAGTGACATAGCAGGAACCTACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCCAGTA

Inact. 6
GGAGAATCTATAAAAGATGGATAATCCTGGGATTAAATAAAATAGTAAGATGTATAGCCCTACCAGCATTCTGGAG
G C G G G C G C G G

ATAAGACAAGGACCAAGGAACCCCTTTAGAGATTATGTAGACCGGTTCTATAAACTCTAAGAGCCCAACAAGCTTCA
T

Inact. 7
CAGGATGTAAAAAATTGGATGACAGAAACCTTGTGGTCCAAAATGCAAAACCAGATTGTAAGACTATTTTAAAGCA
C C C G G T

Inact. 7
TTGGGACCGAGCAGCTACACTAGAGAAATGATGACAGCATGTCAGGGAGTGGGGGGACCCGGCCATAAAGCAAGAGTT
C C C G

Inact. 8
TTGGGTGAAGCCATGAGCCAAAGTAACAAATCCAGCTTACATAATGATGCAGAGAGGCAATTTTAGGAACCAAGAAAG
C G G G G G G C G G C

Inact. 9
ACTGTTAAGTGTCTCAATTGTGGCAAAGAAGGGGCATAGCCAAAATTCAGGGCCCTAGGAAGAGGGCTGTTGG
C C C C C C C C C

AGATGTGAAGGGAAGGACACCAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTAGGGAAGATCTGGCCTTCC

TACAAGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCAAAGCCACCAGAGAGCTTCAGGTTTGGG

GAGGAGAAAACAACTCCCTCTCAGAACGAGGAGCCGATAGACAAGGAAGTGTATCCTTTAACTTCCCTCAGATCACTC

TTTGCAACGACCCCTCGTCACATAAAGGATAGGGGGCAACTAAAGGAAGCTCTATTAGATACAGGAGCAGATGATA
Inact. 11 or only inactivation (GP2)
G C C C C C C C C C C C C C C C C

CAGTATTAGAAGAAATGAATTTGCCAGGAAAATGGAACCAAAAATGATAGGGGGAATTGGAGGTTTATCAAAGTAA
Inact. 12
G C G C C G G

Inact. 12
GACAGTACGATCAGATACCTGTAGAAATCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCA
G C C

ACATAATTGGAAGAAATCTGTTGACTCAGATTGGTTGTACTTAAATTTCCCATTTAGTCTATTGAACTGTACCAG
Inact. 13
C C C C C C C C C C C C C C C C C

Inact. 14
TAAAATTAAAGCCAGGAATGGATGGCCAAAAGTAAAGCAATGGCCATTGTAA
G G G G G C C G C

FIG. 2

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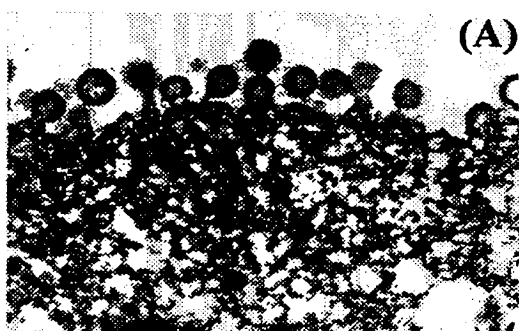


FIG. 3A

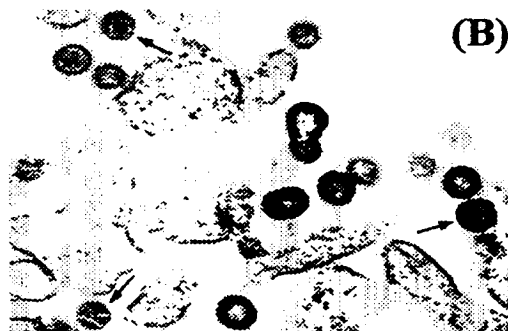


FIG. 3B

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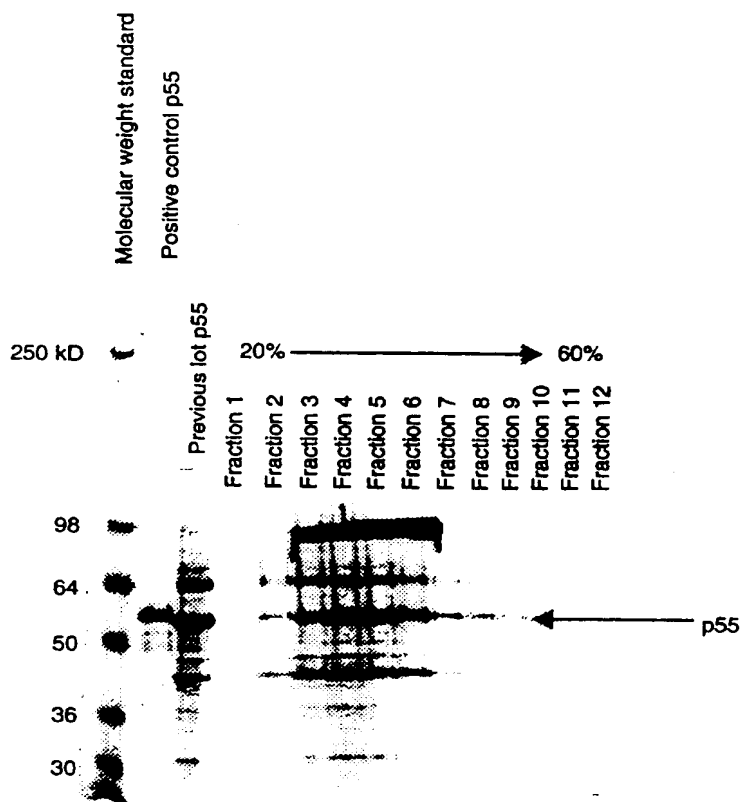


FIG. 4

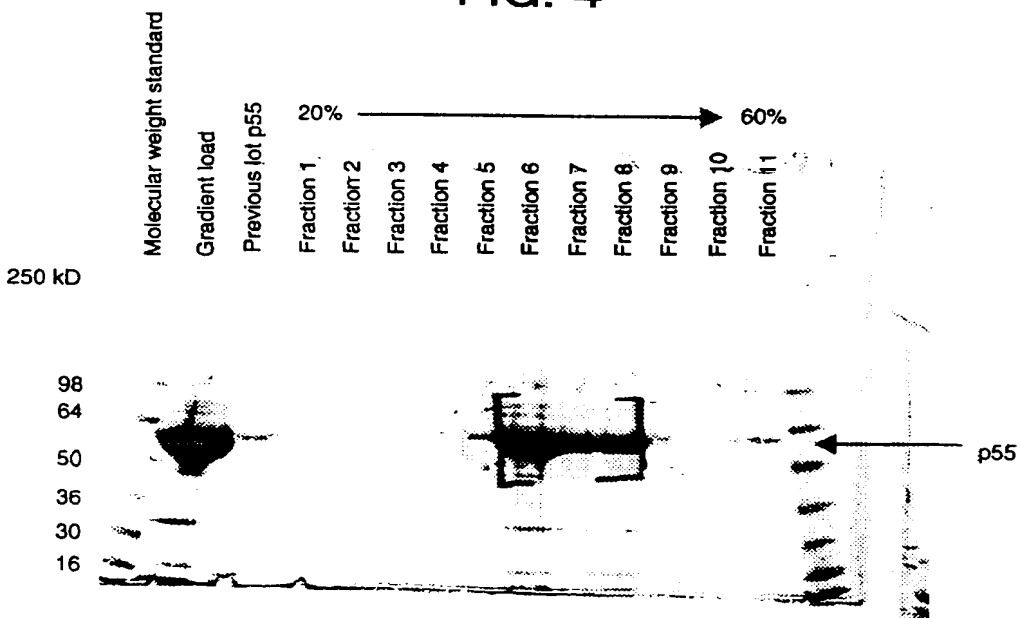


FIG. 5

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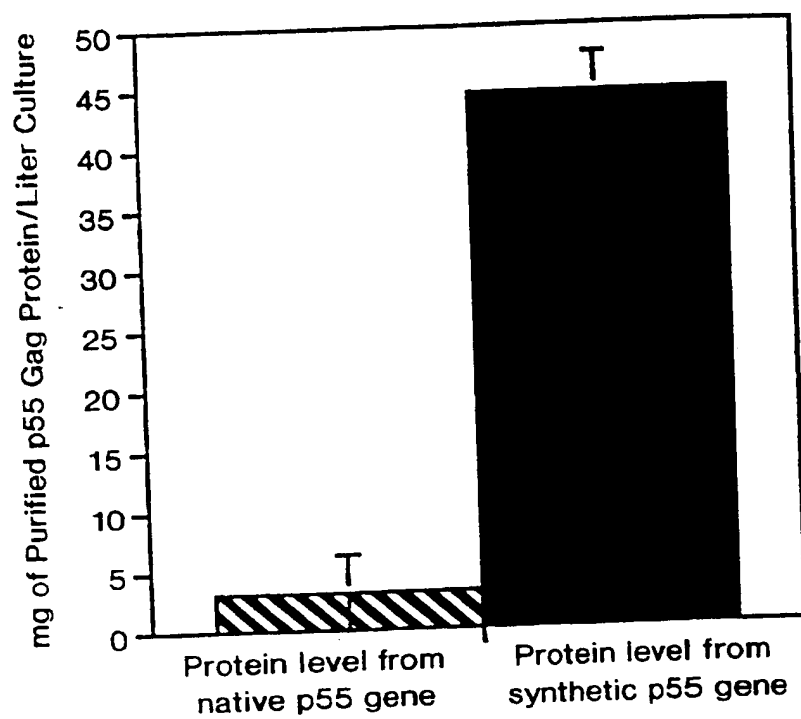


FIG. 6

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| | | | | | | | | | | | |
|--------------|-----|------------|-----|------------|-----|-------------|-----|------------|-----|-------------|-----|
| GagPol.ModSF | 1 | ATGGGCGCCC | 10 | GCGCAGCGT | 20 | GCTGAGCGG | 30 | GGCAGCTGG | 40 | ACAAAGTGGGA | 50 |
| GagProt.Mods | 1 | ATGGGCGCCC | | GCGCAGCGT | | GCTGAGCGG | | GGCAGCTGG | | ACAAAGTGGGA | 50 |
| Gag.ModSF2 | 1 | ATGGGCGCCC | 60 | GCGCAGCGT | 70 | GCTGAGCGG | 80 | GGCAGCTGG | 90 | ACAAAGTGGGA | 100 |
| GagPol.ModSF | 51 | GAAGATCCGC | 110 | CTGGGCCCCG | 120 | GCGCAAGAA | 130 | GAAGTACAAG | 140 | CTGAAGCACA | 150 |
| GagProt.Mods | 51 | GAAGATCCGC | | CTGGGCCCCG | | GCGCAAGAA | | GAAGTACAAG | | CTGAAGCACA | 150 |
| Gag.ModSF2 | 51 | GAAGATCCGC | 110 | CTGGGCCCCG | 120 | GCGCAAGAA | 130 | GAAGTACAAG | 140 | CTGAAGCACA | 150 |
| GagPol.ModSF | 101 | TCGTGTGGG | 160 | CAGCCCGGAG | 170 | CTGAGCGCT | 180 | TCGCCGTGAA | 190 | CCCCGGCCTG | 200 |
| GagProt.Mods | 101 | TCGTGTGGG | | CAGCCCGGAG | | CTGAGCGCT | | TCGCCGTGAA | | CCCCGGCCTG | 200 |
| Gag.ModSF2 | 101 | TCGTGTGGG | 160 | CAGCCCGGAG | 170 | CTGAGCGCT | 180 | TCGCCGTGAA | 190 | CCCCGGCCTG | 200 |
| GagPol.ModSF | 151 | CTGGAGACCA | 210 | GCGAGGGCTG | 220 | CCGCCAGATC | 230 | CTGGGCCAGC | 240 | TGCAGCCCCAG | 250 |
| GagProt.Mods | 151 | CTGGAGACCA | | GCGAGGGCTG | | CCGCCAGATC | | CTGGGCCAGC | | TGCAGCCCCAG | 250 |
| Gag.ModSF2 | 151 | CTGGAGACCA | 210 | GCGAGGGCTG | 220 | CCGCCAGATC | 230 | CTGGGCCAGC | 240 | TGCAGCCCCAG | 250 |
| GagPol.ModSF | 201 | CCTGCAGACC | 260 | GGCAGCGAGG | 270 | AGCTGGCGAG | 280 | CCTGTACAAC | 290 | ACCGTGGCCA | 300 |
| GagProt.Mods | 201 | CCTGCAGACC | | GGCAGCGAGG | | AGCTGGCGAG | | CCTGTACAAC | | ACCGTGGCCA | 300 |
| Gag.ModSF2 | 201 | CCTGCAGACC | 260 | GGCAGCGAGG | 270 | AGCTGGCGAG | 280 | CCTGTACAAC | 290 | ACCGTGGCCA | 300 |
| GagPol.ModSF | 251 | CCCTGTACTG | 310 | CGTGACCCAG | 320 | CGCATCGACG | 330 | TCAAGGACAC | 340 | CAAGGAGGCC | 350 |
| GagProt.Mods | 251 | CCCTGTACTG | | CGTGACCCAG | | CGCATCGACG | | TCAAGGACAC | | CAAGGAGGCC | 350 |
| Gag.ModSF2 | 251 | CCCTGTACTG | 310 | CGTGACCCAG | 320 | CGCATCGACG | 330 | TCAAGGACAC | 340 | CAAGGAGGCC | 350 |
| GagPol.ModSF | 301 | CTGGAGAGA | 360 | TCGAGGAGGA | 370 | GCAGAACAAAG | 380 | TCCAAGAAGA | 390 | AGGCCCCAGCA | 400 |
| GagProt.Mods | 301 | CTGGAGAGA | | TCGAGGAGGA | | GCAGAACAAAG | | TCCAAGAAGA | | AGGCCCCAGCA | 400 |
| Gag.ModSF2 | 301 | CTGGAGAGA | 360 | TCGAGGAGGA | 370 | GCAGAACAAAG | 380 | TCCAAGAAGA | 390 | AGGCCCCAGCA | 400 |
| GagPol.ModSF | 351 | GGCCGCCGCC | 410 | GCCGCCGGCA | 420 | CCGGCAACAG | 430 | CAGCCAGGTG | 440 | AGCCAGAACT | 450 |
| GagProt.Mods | 351 | GGCCGCCGCC | | GCCGCCGGCA | | CCGGCAACAG | | CAGCCAGGTG | | AGCCAGAACT | 450 |
| Gag.ModSF2 | 351 | GGCCGCCGCC | 410 | GCCGCCGGCA | 420 | CCGGCAACAG | 430 | CAGCCAGGTG | 440 | AGCCAGAACT | 450 |
| GagPol.ModSF | 401 | ACCCCATCGT | 460 | GCAGAACCTG | 470 | CAGGGCCAGA | 480 | TGCTGCACCA | 490 | GGCCATCAGC | 500 |
| GagProt.Mods | 401 | ACCCCATCGT | | GCAGAACCTG | | CAGGGCCAGA | | TGCTGCACCA | | GGCCATCAGC | 500 |
| Gag.ModSF2 | 401 | ACCCCATCGT | 460 | GCAGAACCTG | 470 | CAGGGCCAGA | 480 | TGCTGCACCA | 490 | GGCCATCAGC | 500 |

FIG. 7A

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| | | | | | | | |
|--------------|-----|------------|------------|------------|------------|-------------|-----|
| GagPol.ModSF | 451 | CCCCGACCC | TGAACGCCTG | GGTGAAGGTG | GTGGAGGAGA | AGGCTTTCAG | 500 |
| GagProt.ModS | 451 | CCCCGACCC | TGAACGCCTG | GGTGAAGGTG | GTGGAGGAGA | AGGCTTTCAG | 500 |
| Gag.ModSF2 | 451 | CCCCGACCC | TGAACGCCTG | GGTGAAGGTG | GTGGAGGAGA | AGGCTTTCAG | 500 |
| GagPol.ModSF | 501 | CCCCGAGGTG | ATCCCCATGT | TCAGCGCCCT | GAGCGAGGGC | GCCACCCCCC | 550 |
| GagProt.ModS | 501 | CCCCGAGGTG | ATCCCCATGT | TCAGCGCCCT | GAGCGAGGGC | GCCACCCCCC | 550 |
| Gag.ModSF2 | 501 | CCCCGAGGTG | ATCCCCATGT | TCAGCGCCCT | GAGCGAGGGC | GCCACCCCCC | 550 |
| GagPol.ModSF | 551 | AGGACCTGAA | CACGATGTTG | AACACCGTGG | GCGGCCACCA | GGCCGCCATG | 600 |
| GagProt.ModS | 551 | AGGACCTGAA | CACGATGTTG | AACACCGTGG | GCGGCCACCA | GGCCGCCATG | 600 |
| Gag.ModSF2 | 551 | AGGACCTGAA | CACGATGTTG | AACACCGTGG | GCGGCCACCA | GGCCGCCATG | 600 |
| GagPol.ModSF | 601 | CAGATGCTGA | AGGAGACCAT | CAACGAGGAG | GCCGCCGAGT | GGGACCGCGT | 650 |
| GagProt.ModS | 601 | CAGATGCTGA | AGGAGACCAT | CAACGAGGAG | GCCGCCGAGT | GGGACCGCGT | 650 |
| Gag.ModSF2 | 601 | CAGATGCTGA | AGGAGACCAT | CAACGAGGAG | GCCGCCGAGT | GGGACCGCGT | 650 |
| GagPol.ModSF | 651 | GCACCCCGTG | CACGCCGGCC | CCATCGCCCC | CGGCCAGATG | CGCGAGCCCC | 700 |
| GagProt.ModS | 651 | GCACCCCGTG | CACGCCGGCC | CCATCGCCCC | CGGCCAGATG | CGCGAGCCCC | 700 |
| Gag.ModSF2 | 651 | GCACCCCGTG | CACGCCGGCC | CCATCGCCCC | CGGCCAGATG | CGCGAGCCCC | 700 |
| GagPol.ModSF | 701 | GCGGCAGCGA | CATCGCCGGC | ACCACACGCA | CCCTGCAGGA | GCAGATCGGC | 750 |
| GagProt.ModS | 701 | GCGGCAGCGA | CATCGCCGGC | ACCACACGCA | CCCTGCAGGA | GCAGATCGGC | 750 |
| Gag.ModSF2 | 701 | GCGGCAGCGA | CATCGCCGGC | ACCACACGCA | CCCTGCAGGA | GCAGATCGGC | 750 |
| GagPol.ModSF | 751 | TGGATGACCA | ACAAACCCCC | CATCCCCCGT | GGCGAGATCT | ACAAAGCGGTG | 800 |
| GagProt.ModS | 751 | TGGATGACCA | ACAAACCCCC | CATCCCCCGT | GGCGAGATCT | ACAAAGCGGTG | 800 |
| Gag.ModSF2 | 751 | TGGATGACCA | ACAAACCCCC | CATCCCCCGT | GGCGAGATCT | ACAAAGCGGTG | 800 |
| GagPol.ModSF | 801 | GATCATCCTG | GGCCTGAACA | AGATCGTGCG | GATGTACAGC | CCCACACGCA | 850 |
| GagProt.ModS | 801 | GATCATCCTG | GGCCTGAACA | AGATCGTGCG | GATGTACAGC | CCCACACGCA | 850 |
| Gag.ModSF2 | 801 | GATCATCCTG | GGCCTGAACA | AGATCGTGCG | GATGTACAGC | CCCACACGCA | 850 |
| GagPol.ModSF | 851 | TCCTGGACAT | CCGCCAGGGC | CCCAGGAGC | CCTTCCCGCA | CTACGTGGAC | 900 |
| GagProt.ModS | 851 | TCCTGGACAT | CCGCCAGGGC | CCCAGGAGC | CCTTCCCGCA | CTACGTGGAC | 900 |
| Gag.ModSF2 | 851 | TCCTGGACAT | CCGCCAGGGC | CCCAGGAGC | CCTTCCCGCA | CTACGTGGAC | 900 |

FIG. 7B

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| | | | | | | | | | | | |
|--------------|------|------------|------|-------------|------|------------|------|------------|------|------------|------|
| GagPol.ModSF | 901 | CGCTTCTACA | 910 | AGACCTCTGC | 920 | CGCTGAGCAG | 930 | GCCAGCCAGG | 940 | ACGTGAAGAA | 950 |
| GagProt.ModS | 901 | CGCTTCTACA | 910 | AGACCTCTGC | 920 | CGCTGAGCAG | 930 | GCCAGCCAGG | 940 | ACGTGAAGAA | 950 |
| Gag.ModSF2 | 901 | CGCTTCTACA | 910 | AGACCTCTGC | 920 | CGCTGAGCAG | 930 | GCCAGCCAGG | 940 | ACGTGAAGAA | 950 |
| GagPol.ModSF | 951 | CTGGATGACC | 960 | GAGACCTCTGC | 970 | TGGTGCAGAA | 980 | CGCCAAACCC | 990 | GACTGCAAGA | 1000 |
| GagProt.ModS | 951 | CTGGATGACC | 960 | GAGACCTCTGC | 970 | TGGTGCAGAA | 980 | CGCCAAACCC | 990 | GACTGCAAGA | 1000 |
| Gag.ModSF2 | 951 | CTGGATGACC | 960 | GAGACCTCTGC | 970 | TGGTGCAGAA | 980 | CGCCAAACCC | 990 | GACTGCAAGA | 1000 |
| GagPol.ModSF | 1001 | CCATCCTGAA | 1010 | GGCTCTCGGC | 1020 | CCCGGGGCGA | 1030 | CCCTGGAGGA | 1040 | GATGATGACC | 1050 |
| GagProt.ModS | 1001 | CCATCCTGAA | 1010 | GGCTCTCGGC | 1020 | CCCGGGGCGA | 1030 | CCCTGGAGGA | 1040 | GATGATGACC | 1050 |
| Gag.ModSF2 | 1001 | CCATCCTGAA | 1010 | GGCTCTCGGC | 1020 | CCCGGGGCGA | 1030 | CCCTGGAGGA | 1040 | GATGATGACC | 1050 |
| GagPol.ModSF | 1051 | GCCTGCCAGG | 1060 | GCGTGGGCGG | 1070 | CCCCGGCCAC | 1080 | AAGGCCCGCG | 1090 | TGCTGGCCGA | 1100 |
| GagProt.ModS | 1051 | GCCTGCCAGG | 1060 | GCGTGGGCGG | 1070 | CCCCGGCCAC | 1080 | AAGGCCCGCG | 1090 | TGCTGGCCGA | 1100 |
| Gag.ModSF2 | 1051 | GCCTGCCAGG | 1060 | GCGTGGGCGG | 1070 | CCCCGGCCAC | 1080 | AAGGCCCGCG | 1090 | TGCTGGCCGA | 1100 |
| GagPol.ModSF | 1101 | GCGGATGAGC | 1110 | CAGGTGACGA | 1120 | ACCCGGCGAC | 1130 | CATCATGATG | 1140 | CAGCGCGGCA | 1150 |
| GagProt.ModS | 1101 | GCGGATGAGC | 1110 | CAGGTGACGA | 1120 | ACCCGGCGAC | 1130 | CATCATGATG | 1140 | CAGCGCGGCA | 1150 |
| Gag.ModSF2 | 1101 | GCGGATGAGC | 1110 | CAGGTGACGA | 1120 | ACCCGGCGAC | 1130 | CATCATGATG | 1140 | CAGCGCGGCA | 1150 |
| GagPol.ModSF | 1151 | ACTTCCGCAA | 1160 | CCAGCGGAAG | 1170 | ACCGTCAAGT | 1180 | GCTTCAACTG | 1190 | CGGCAAGGAG | 1200 |
| GagProt.ModS | 1151 | ACTTCCGCAA | 1160 | CCAGCGGAAG | 1170 | ACCGTCAAGT | 1180 | GCTTCAACTG | 1190 | CGGCAAGGAG | 1200 |
| Gag.ModSF2 | 1151 | ACTTCCGCAA | 1160 | CCAGCGGAAG | 1170 | ACCGTCAAGT | 1180 | GCTTCAACTG | 1190 | CGGCAAGGAG | 1200 |
| GagPol.ModSF | 1201 | GGCCACACCG | 1210 | CCAGGAACTG | 1220 | CCGCGCCCCC | 1230 | CGCAAGAAGG | 1240 | GCTGCTGGCG | 1250 |
| GagProt.ModS | 1201 | GGCCACACCG | 1210 | CCAGGAACTG | 1220 | CCGCGCCCCC | 1230 | CGCAAGAAGG | 1240 | GCTGCTGGCG | 1250 |
| Gag.ModSF2 | 1201 | GGCCACACCG | 1210 | CCAGGAACTG | 1220 | CCGCGCCCCC | 1230 | CGCAAGAAGG | 1240 | GCTGCTGGCG | 1250 |
| GagPol.ModSF | 1251 | CTGCGGCCGC | 1260 | GAAGGACACC | 1270 | AAATGAAAGA | 1280 | TTGCACTGAG | 1290 | AGACAGGCTA | 1300 |
| GagProt.ModS | 1251 | CTGCGGCCGC | 1260 | GAAGGACACC | 1270 | AAATGAAAGA | 1280 | TTGCACTGAG | 1290 | AGACAGGCTA | 1300 |
| Gag.ModSF2 | 1251 | CTGCGGCCGC | 1260 | GAAGGACACC | 1270 | AAATGAAAGA | 1280 | TTGCACTGAG | 1290 | AGACAGGCTA | 1300 |
| GagPol.ModSF | 1301 | ATTTTCTAGG | 1310 | GAAGATCTGG | 1320 | CCTTCCTACA | 1330 | AGGGAAGGCC | 1340 | AGGGAATTTT | 1350 |
| GagProt.ModS | 1301 | ATTTTCTAGG | 1310 | GAAGATCTGG | 1320 | CCTTCCTACA | 1330 | AGGGAAGGCC | 1340 | AGGGAATTTT | 1350 |
| Gag.ModSF2 | 1301 | ATTTTCTAGG | 1310 | GAAGATCTGG | 1320 | CCTTCCTACA | 1330 | AGGGAAGGCC | 1340 | AGGGAATTTT | 1350 |

FIG. 7C

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| | | | | | | | | | | | |
|--------------|------|-------------|------|-------------|------|------------|------|------------|------|-------------|------|
| GagPol.ModSF | 1351 | CTTCAGAGCA | 1360 | GACCAGAGCC | 1370 | AACAGCCCCA | 1380 | CCAGAAGAGA | 1390 | GCTTCAGGTT | 1400 |
| GagProt.ModS | 1351 | CTTCAGAGCA | | GACCAGAGCC | | AACAGCCCCA | | CCAGAAGAGA | | GCTTCAGGTT | 1400 |
| Gag.ModSF2 | 1351 | CTGCAGAGCC | 1410 | GCCCCGAGCC | 1420 | CACCGCCCCC | 1430 | CCCGAGGAGA | 1440 | GCTTCGCGTT | 1450 |
| GagPol.ModSF | 1401 | TGGGGAGGAG | 1460 | AAAACAACCTC | 1470 | CCTCTCAGAA | 1480 | GCAGGAGCCG | 1490 | ATAGACAAGG | 1500 |
| GagProt.ModS | 1401 | TGGGGAGGAG | | AAAACAACCTC | | CCTCTCAGAA | | GCAGGAGCCG | | ATAGACAAGG | 1500 |
| Gag.ModSF2 | 1401 | CGGCGAGGAG | 1460 | AAGACCACCC | 1470 | CCAGCCAGAA | 1480 | GCAGGAGCCG | 1490 | ATCGACAAGG | 1500 |
| GagPol.ModSF | 1451 | AACTGTATCC | 1510 | TTTAACTTCC | 1520 | CTCAGATCAC | 1530 | TCITTGGCAA | 1540 | CGACCCCTCG | 1550 |
| GagProt.ModS | 1451 | AACTGTATCC | | TTTAACTTCC | | CTCAGATCAC | | TCITTGGCAA | | CGACCCCTCG | 1550 |
| Gag.ModSF2 | 1451 | AGCTGTATCC | 1510 | CCTGACCAGC | 1520 | CTGCGGAGCC | 1530 | TGTTCCGCAA | 1540 | CGACCCCTCG | 1550 |
| GagPol.ModSF | 1501 | TCACAGTAAG | 1560 | GATCGGCGGC | 1570 | CAGCTCAAGG | 1580 | AGGCGTGCT | 1590 | CGACACCGGC | 1600 |
| GagProt.ModS | 1501 | TCACAGTAAG | | GATCGGCGGC | | CAGCTCAAGG | | AGGCGTGCT | | CGACACCGGC | 1600 |
| Gag.ModSF2 | 1501 | AGCCAGTAA | 1560 | | 1570 | | 1580 | | 1590 | | 1600 |
| GagPol.ModSF | 1551 | GCCGACGACA | 1610 | CCGTGCTGGA | 1620 | GGAGATGAAC | 1630 | CTGCCCGGCA | 1640 | AGTGGAAAGCC | 1650 |
| GagProt.ModS | 1551 | GCCGACGACA | | CCGTGCTGGA | | GGAGATGAAC | | CTGCCCGGCA | | AGTGGAAAGCC | 1650 |
| Gag.ModSF2 | 1551 | | 1610 | | 1620 | | 1630 | | 1640 | | 1650 |
| GagPol.ModSF | 1601 | CAAGATGATC | 1660 | GGCGGGATCG | 1670 | GGGGCTTCAT | 1680 | CAAGGTGCGG | 1690 | CAGTACGACC | 1700 |
| GagProt.ModS | 1601 | CAAGATGATC | | GGCGGGATCG | | GGGGCTTCAT | | CAAGGTGCGG | | CAGTACGACC | 1700 |
| Gag.ModSF2 | 1601 | | 1660 | | 1670 | | 1680 | | 1690 | | 1700 |
| GagPol.ModSF | 1651 | AGATCCCCCGT | 1710 | GGAGATCTGC | 1720 | GGCCACAAGG | 1730 | CCATCGGCAC | 1740 | CGTGCTGGTG | 1750 |
| GagProt.ModS | 1651 | AGATCCCCCGT | | GGAGATCTGC | | GGCCACAAGG | | CCATCGGCAC | | CGTGCTGGTG | 1750 |
| Gag.ModSF2 | 1651 | | 1710 | | 1720 | | 1730 | | 1740 | | 1750 |
| GagPol.ModSF | 1701 | GGCCCCACCC | 1760 | CCGTGAACAT | 1770 | CATCGGCCGC | 1780 | AACCTGCTGA | 1790 | CCCAGATCGG | 1800 |
| GagProt.ModS | 1701 | GGCCCCACCC | | CCGTGAACAT | | CATCGGCCGC | | AACCTGCTGA | | CCCAGATCGG | 1800 |
| Gag.ModSF2 | 1701 | | 1760 | | 1770 | | 1780 | | 1790 | | 1800 |
| GagPol.ModSF | 1751 | CTGCACCCCTG | 1800 | AACCTTCCCA | 1810 | TCAGCCCAT | 1820 | CGAGACGGTG | 1830 | CCCGTGAAGC | 1840 |
| GagProt.ModS | 1751 | CTGCACCCCTG | | AACCTTCCCA | | TCAGCCCAT | | CGAGACGGTG | | CCCGTGAAGC | 1840 |
| Gag.ModSF2 | 1751 | | 1800 | | 1810 | | 1820 | | 1830 | | 1840 |

FIG. 7D

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FIG. 7E

| | | | | | | |
|--------------|------|------------|-------------|------------|------------|------------|
| GagPol.ModSF | 1801 | 1810 | 1820 | 1830 | 1840 | 1850 |
| GagProt.ModS | 1801 | TGAAGCCGGG | GATGGACGGC | CCCAAGGTCA | AGCAGTGGCC | CCTGACCGAG |
| Gag.ModSF2 | 1801 | TGAAGCCGGG | GATGGACGGC | CCCAAGGTCA | AGCAGTGGCC | CCTGTAA... |
| | | 1860 | 1870 | 1880 | 1890 | 1900 |
| GagPol.ModSF | 1851 | GAGAAGATCA | AGGCCCTGGT | GGAGATCTGC | ACCGAGATGG | AGAAGGAGGG |
| GagProt.ModS | 1851 | | | | | |
| Gag.ModSF2 | 1851 | | | | | |
| | | 1910 | 1920 | 1930 | 1940 | 1950 |
| GagPol.ModSF | 1901 | CAAGATCAGC | AAGATCGGCC | CCGAGAATCC | CTACAACACC | CCCGTGTTCG |
| GagProt.ModS | 1901 | | | | | |
| Gag.ModSF2 | 1901 | | | | | |
| | | 1960 | 1970 | 1980 | 1990 | 2000 |
| GagPol.ModSF | 1951 | CCATCAAGAA | GAAGGACAGC | ACCAAGTGGC | GCAAGCTGGT | GGACTTCCGC |
| GagProt.ModS | 1951 | | | | | |
| Gag.ModSF2 | 1951 | | | | | |
| | | 2010 | 2020 | 2030 | 2040 | 2050 |
| GagPol.ModSF | 2001 | GAGCTGAACA | AGCGCACCCA | GGACTTCTGG | GAGGTGCAGC | TGGGCATCCC |
| GagProt.ModS | 2001 | | | | | |
| Gag.ModSF2 | 2001 | | | | | |
| | | 2060 | 2070 | 2080 | 2090 | 2100 |
| GagPol.ModSF | 2051 | CCACCCCGCC | GGCCTGAAGA | AGAAGAAGAG | CGTGACCGTG | CTGGACCTGG |
| GagProt.ModS | 2051 | | | | | |
| Gag.ModSF2 | 2051 | | | | | |
| | | 2110 | 2120 | 2130 | 2140 | 2150 |
| GagPol.ModSF | 2101 | GCGACGCCTA | CTTCAGCGTG | CCCCTGGACA | AGGACTTCCG | CAAGTACACC |
| GagProt.ModS | 2101 | | | | | |
| Gag.ModSF2 | 2101 | | | | | |
| | | 2160 | 2170 | 2180 | 2190 | 2200 |
| GagPol.ModSF | 2151 | GCCTTCACCA | TCCCCAGCAT | CAACAACGAG | ACCCCCGGCA | TCCGCTACCA |
| GagProt.ModS | 2151 | | | | | |
| Gag.ModSF2 | 2151 | | | | | |
| | | 2210 | 2220 | 2230 | 2240 | 2250 |
| GagPol.ModSF | 2201 | GTACAACGTG | CTGCCCCCAGG | GCTGGAAGGG | CAGCCCCGGC | ATCTTCCAGA |
| GagProt.ModS | 2201 | | | | | |
| Gag.ModSF2 | 2201 | | | | | |

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| | | | | | |
|-------------------|-------------|------------|------------|-------------|-------------|
| GagPol.ModSF 2251 | 2260 | 2270 | 2280 | 2290 | 2300 |
| GagProt.Mods 2251 | GCAGCATGAC | CAAGATCCTG | GAGCCCTTCC | GCAAGCAGAA | CCCCGACATC |
| Gag.ModSF2 2251 | | | | | |
| | 2310 | 2320 | 2330 | 2340 | 2350 |
| GagPol.ModSF 2301 | GTGATCTACC | AGTACATGGA | CGACCTGTAC | GTGGGCAGCG | ACCTGGAGAT |
| GagProt.Mods 2301 | | | | | |
| Gag.ModSF2 2301 | | | | | |
| | 2360 | 2370 | 2380 | 2390 | 2400 |
| GagPol.ModSF 2351 | CGGCCAGCAC | CGCACCAAGA | TCGAGGAGCT | GCGCCAGCAC | CTGCTGGGCT |
| GagProt.Mods 2351 | | | | | |
| Gag.ModSF2 2351 | | | | | |
| | 2410 | 2420 | 2430 | 2440 | 2450 |
| GagPol.ModSF 2401 | GGGGCTTCAC | CACCCCGGAC | AAGAAGCACC | AGAAGGAGCC | CCCCCTTCCTG |
| GagProt.Mods 2401 | | | | | |
| Gag.ModSF2 2401 | | | | | |
| | 2460 | 2470 | 2480 | 2490 | 2500 |
| GagPol.ModSF 2451 | TGGATGGGCT | ACGAGCTGCA | CCCCGACAAG | TGGACCGTGC | AGCCCATCAT |
| GagProt.Mods 2451 | | | | | |
| Gag.ModSF2 2451 | | | | | |
| | 2510 | 2520 | 2530 | 2540 | 2550 |
| GagPol.ModSF 2501 | GCTGCCCCGAG | AAGGACAGCT | GGACCGTGAA | CGACATCCAG | AAGCTGGTGG |
| GagProt.Mods 2501 | | | | | |
| Gag.ModSF2 2501 | | | | | |
| | 2560 | 2570 | 2580 | 2590 | 2600 |
| GagPol.ModSF 2551 | GCAAGCTGAA | CTGGGCCAGC | CAGATCTACG | CCGGCATCAA | GGTGAAGCAG |
| GagProt.Mods 2551 | | | | | |
| Gag.ModSF2 2551 | | | | | |
| | 2610 | 2620 | 2630 | 2640 | 2650 |
| GagPol.ModSF 2601 | CTGTGCAAGC | TGCTGGCGGG | CACCAAGGCC | CTGACCCGAGG | TGATCCCCCT |
| GagProt.Mods 2601 | | | | | |
| Gag.ModSF2 2601 | | | | | |
| | 2660 | 2670 | 2680 | 2690 | 2700 |
| GagPol.ModSF 2651 | GACCCGAGGAG | GCCGAGCTGG | AGCTGGCCGA | GAACCCGAGG | ATCCTGAAGG |
| GagProt.Mods 2651 | | | | | |
| Gag.ModSF2 2651 | | | | | |

FIG. 7F

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| | | | | | |
|-------------------|-------------|-------------|------------|------------|-------------|
| GagPol.ModSF 2701 | 2710 | 2720 | 2730 | 2740 | 2750 |
| | AGCCCGTGCA | CGAGGTGTAC | TACGACCCCA | GCAAGGACCT | GGTGGCCGAG |
| GagProt.Mods 2701 | | | | | |
| Gag.ModSF2 2701 | | | | | |
| | 2760 | 2770 | 2780 | 2790 | 2800 |
| GagPol.ModSF 2751 | ATCCAGAAGC | AGGGCCAGGG | CCAGTGGACC | TACCAGATCT | ACCAGGAGCC |
| GagProt.Mods 2751 | | | | | |
| Gag.ModSF2 2751 | | | | | |
| | 2810 | 2820 | 2830 | 2840 | 2850 |
| GagPol.ModSF 2801 | CTTCAAGAAC | CTGAAGACCG | GCAAATACGC | CCGATGCGC | GGCGCCACAC |
| GagProt.Mods 2801 | | | | | |
| Gag.ModSF2 2801 | | | | | |
| | 2860 | 2870 | 2880 | 2890 | 2900 |
| GagPol.ModSF 2851 | CCAACGACGT | GAAGCAGCTG | ACCGAGGCCG | TGCAGAAGGT | GAGCACCCGAG |
| GagProt.Mods 2851 | | | | | |
| Gag.ModSF2 2851 | | | | | |
| | 2910 | 2920 | 2930 | 2940 | 2950 |
| GagPol.ModSF 2901 | AGCATCGTGA | TCTGGGGCAA | GATCCCCAAG | TTCAAGCTGC | CCATCCAGAA |
| GagProt.Mods 2901 | | | | | |
| Gag.ModSF2 2901 | | | | | |
| | 2960 | 2970 | 2980 | 2990 | 3000 |
| GagPol.ModSF 2951 | GGAGACCTGG | GAGGCCTGGT | GGATGGAGTA | CTGGCAGGCC | ACCTGGATCC |
| GagProt.Mods 2951 | | | | | |
| Gag.ModSF2 2951 | | | | | |
| | 3010 | 3020 | 3030 | 3040 | 3050 |
| GagPol.ModSF 3001 | CCGAGTGGGA | GTTCTGTGAAC | ACCCCCCCCC | TGGTGAAGCT | GTGGTACCAG |
| GagProt.Mods 3001 | | | | | |
| Gag.ModSF2 3001 | | | | | |
| | 3060 | 3070 | 3080 | 3090 | 3100 |
| GagPol.ModSF 3051 | CTGGAGAAGG | AGCCCATCGT | GGGCGCCGAG | ACCTTCTACG | TGGACGGCGC |
| GagProt.Mods 3051 | | | | | |
| Gag.ModSF2 3051 | | | | | |
| | 3110 | 3120 | 3130 | 3140 | 3150 |
| GagPol.ModSF 3101 | CGCCCAACCGC | GAGACCAAGC | TGGGCAAGGC | CGGCTACGTG | ACCGACCGCG |
| GagProt.Mods 3101 | | | | | |
| Gag.ModSF2 3101 | | | | | |

FIG. 7G

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| | | | | | | |
|--------------|------|-------------|-------------|------------|------------|-------------|
| GagPol.ModSF | 3151 | 3160 | 3170 | 3180 | 3190 | 3200 |
| GagProt.Mods | 3151 | GGCGCCAGAA | GGTGTGAGC | ATCGCCGACA | CCACCAACCA | GAAGACCGAG |
| Gag.ModSF2 | 3151 | | | | | |
| GagPol.ModSF | 3201 | 3210 | 3220 | 3230 | 3240 | 3250 |
| GagProt.Mods | 3201 | CTGCAGGCCA | TCCACCTGGC | CCTGCAGGAC | AGCGGCCTGG | AGGTGAACAT |
| Gag.ModSF2 | 3201 | | | | | |
| GagPol.ModSF | 3251 | 3260 | 3270 | 3280 | 3290 | 3300 |
| GagProt.Mods | 3251 | CGTGACCGAC | AGCCAGTACG | CCCTGGGCAT | CATCCAGGCC | CAGCCCCGACA |
| Gag.ModSF2 | 3251 | | | | | |
| GagPol.ModSF | 3301 | 3310 | 3320 | 3330 | 3340 | 3350 |
| GagProt.Mods | 3301 | AGAGCGGAGG | CGAGCTGGTG | AGCCAGATCA | TCGAGCAGCT | GATCAAGAAG |
| Gag.ModSF2 | 3301 | | | | | |
| GagPol.ModSF | 3351 | 3360 | 3370 | 3380 | 3390 | 3400 |
| GagProt.Mods | 3351 | GAGAAGGTGT | ACCTGGCCTG | GGTGCCCGCC | CACAAAGGCA | TCGGCGGCAA |
| Gag.ModSF2 | 3351 | | | | | |
| GagPol.ModSF | 3401 | 3410 | 3420 | 3430 | 3440 | 3450 |
| GagProt.Mods | 3401 | CGAGCAGGTG | GACAAGCTGG | TGAGCGCCGG | CATCCCGAAG | GTGCTGTTC |
| Gag.ModSF2 | 3401 | | | | | |
| GagPol.ModSF | 3451 | 3460 | 3470 | 3480 | 3490 | 3500 |
| GagProt.Mods | 3451 | TGAAACGGCAT | CGACAAAGGCC | CAGGAGGAGC | ACGAGAAGTA | CCACAGGCAAC |
| Gag.ModSF2 | 3451 | | | | | |
| GagPol.ModSF | 3501 | 3510 | 3520 | 3530 | 3540 | 3550 |
| GagProt.Mods | 3501 | TGGCGCGCCA | TGGCCAGCGA | CTTCAACCTG | CCCCCGTGG | TGGCCCAAGGA |
| Gag.ModSF2 | 3501 | | | | | |
| GagPol.ModSF | 3551 | 3560 | 3570 | 3580 | 3590 | 3600 |
| GagProt.Mods | 3551 | GATCGTGGCC | AGCTGGGACA | AGTGCCAGCT | GAAGGGCGAG | GCCATGCACG |
| Gag.ModSF2 | 3551 | | | | | |

FIG. 7H

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| | | | | | |
|-------------------|------------|------------|------------|-------------|-------------|
| GagPol.ModSF 3601 | 3610 | 3620 | 3630 | 3640 | 3650 |
| GagProt.Mods 3601 | GCCAGGTGGA | CTGCAGCCCC | GGCATCTGGC | AGCTGGACTG | CACCCACCTG |
| Gag.ModSF2 3601 | | | | | |
| GagPol.ModSF 3651 | 3660 | 3670 | 3680 | 3690 | 3700 |
| GagProt.Mods 3651 | GAGGGCAAGA | TCATCCTGGT | GGCCGTGCAC | GTGGCCAGCG | GCTACATCGA |
| Gag.ModSF2 3651 | | | | | |
| GagPol.ModSF 3701 | 3710 | 3720 | 3730 | 3740 | 3750 |
| GagProt.Mods 3701 | GGCCGAGGTG | ATCCCCGCGG | AGACCGGCCA | GGAGACCGCC | TACTTCCTGC |
| Gag.ModSF2 3701 | | | | | |
| GagPol.ModSF 3751 | 3760 | 3770 | 3780 | 3790 | 3800 |
| GagProt.Mods 3751 | TGAAGCTGGC | CGGCCGCTGG | CCCGTGAAGA | CCATCCACAC | CGACAACGGC |
| Gag.ModSF2 3751 | | | | | |
| GagPol.ModSF 3801 | 3810 | 3820 | 3830 | 3840 | 3850 |
| GagProt.Mods 3801 | AGCAACTTCA | CCAGCACACC | CGTGAAGGCC | GCCTGCTGGT | GGGCCGGGCAT |
| Gag.ModSF2 3801 | | | | | |
| GagPol.ModSF 3851 | 3860 | 3870 | 3880 | 3890 | 3900 |
| GagProt.Mods 3851 | CAAGCAGGAG | TTCGGCATCC | CCTACAACCC | CCAGAGCCAG | GGCGTGGTGG |
| Gag.ModSF2 3851 | | | | | |
| GagPol.ModSF 3901 | 3910 | 3920 | 3930 | 3940 | 3950 |
| GagProt.Mods 3901 | AGAGCATGAA | CAACGAGGTG | AAGAAGATCA | TCGGCCAGGT | GGCGGACCAG |
| Gag.ModSF2 3901 | | | | | |
| GagPol.ModSF 3951 | 3960 | 3970 | 3980 | 3990 | 4000 |
| GagProt.Mods 3951 | GCCGAGCACC | TGAAGACCGC | CGTGCAGATG | GCCGTGTTCA | TCCACAACCTT |
| Gag.ModSF2 3951 | | | | | |
| GagPol.ModSF 4001 | 4010 | 4020 | 4030 | 4040 | 4050 |
| GagProt.Mods 4001 | CAAGGGCAAG | GGCGGCATCG | GCGGCTACAG | CGCCGGCCGAG | CGCATCGTGG |
| Gag.ModSF2 4001 | | | | | |

FIG. 71

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| | | | | | | | |
|--------------|------|-------------|-------|-------|-------|-------|------|
| GagPol.ModSF | 4051 | ACATCATCGC | 4060 | 4070 | 4080 | 4090 | 4100 |
| GagProt.ModS | 4051 | | | | | | 4100 |
| Gag.ModSF2 | 4051 | | | | | | 4100 |
| GagPol.ModSF | 4101 | AAGATCCAGA | 4110 | 4120 | 4130 | 4140 | 4150 |
| GagProt.ModS | 4101 | | | | | | 4150 |
| Gag.ModSF2 | 4101 | | | | | | 4150 |
| GagPol.ModSF | 4151 | GAAGGGCCCC | 4160 | 4170 | 4180 | 4190 | 4200 |
| GagProt.ModS | 4151 | | | | | | 4200 |
| Gag.ModSF2 | 4151 | | | | | | 4200 |
| GagPol.ModSF | 4201 | AGGACAAACAG | 4210 | 4220 | 4230 | 4240 | 4250 |
| GagProt.ModS | 4201 | | | | | | 4250 |
| Gag.ModSF2 | 4201 | | | | | | 4250 |
| GagPol.ModSF | 4251 | CGCGACTAGG | 4260 | 4270 | 4280 | 4290 | 4300 |
| GagProt.ModS | 4251 | | | | | | 4300 |
| Gag.ModSF2 | 4251 | | | | | | 4300 |
| GagPol.ModSF | 4301 | GGACGAGGAC | 4310 | 4320 | 4330 | 4340 | 4350 |
| GagProt.ModS | 4301 | | | | | | 4350 |
| Gag.ModSF2 | 4301 | | | | | | 4350 |

FIG. 7J

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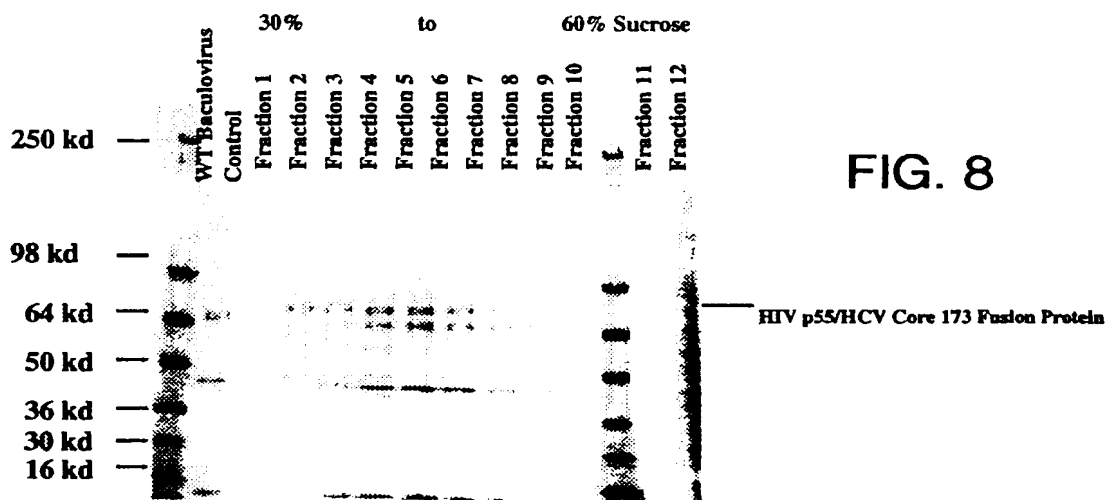


FIG. 8

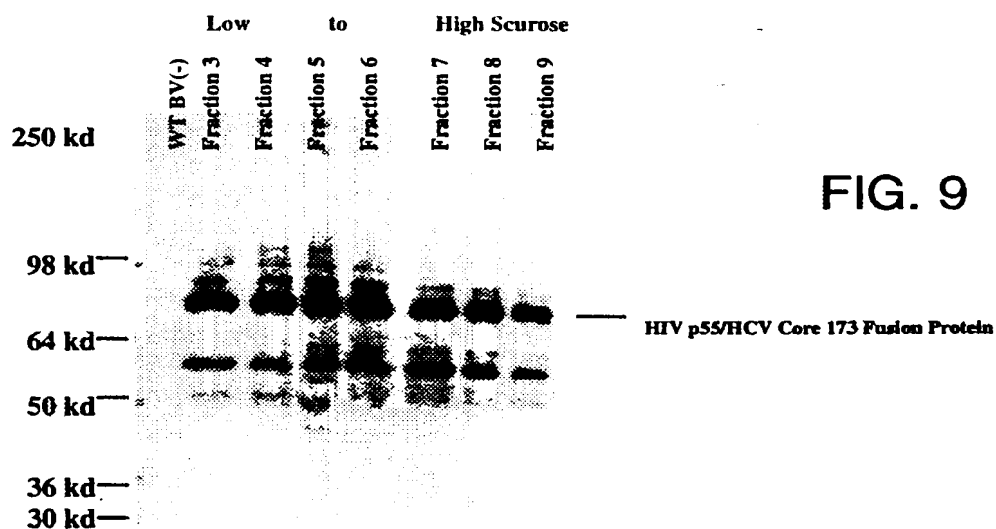


FIG. 9

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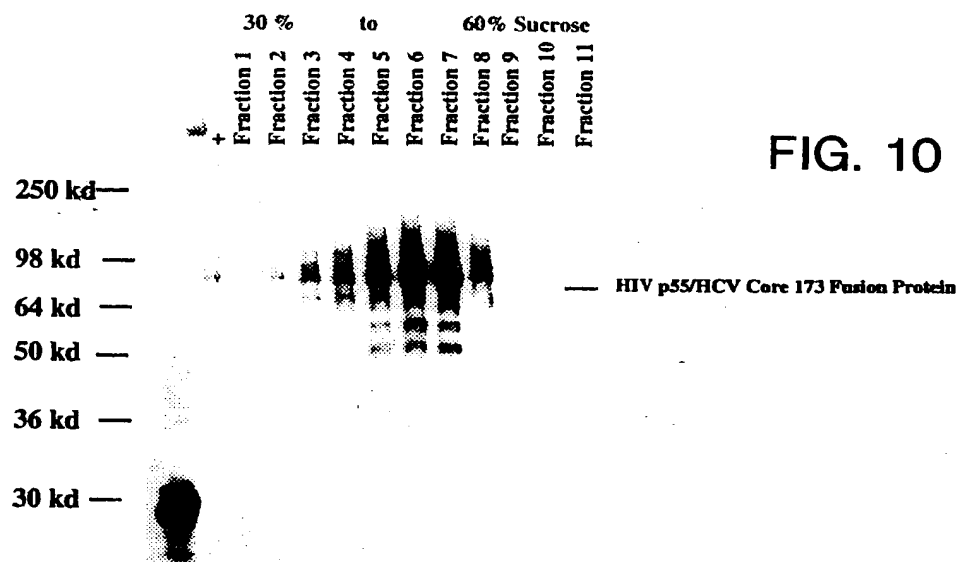


FIG. 10

SUBSTITUTE SHEET (RULE 26)

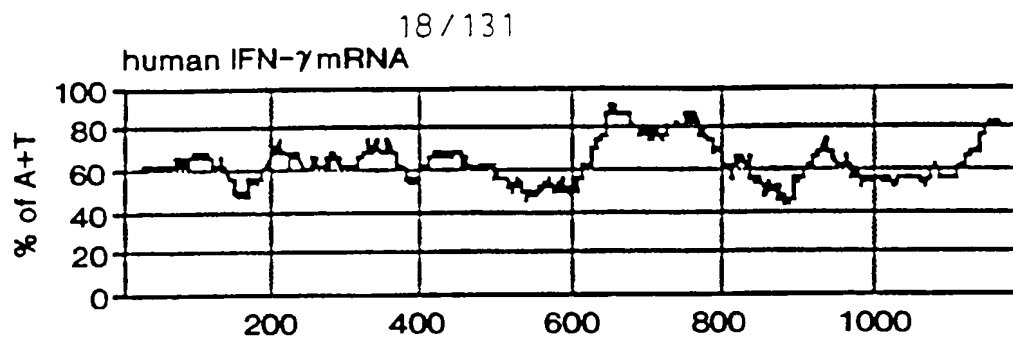


FIG. 11A

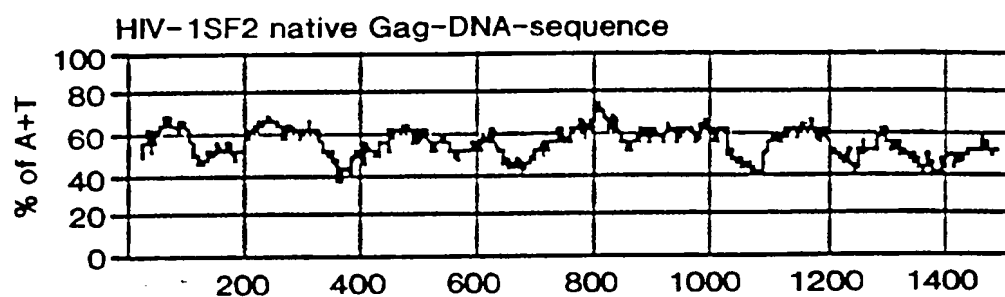


FIG. 11B

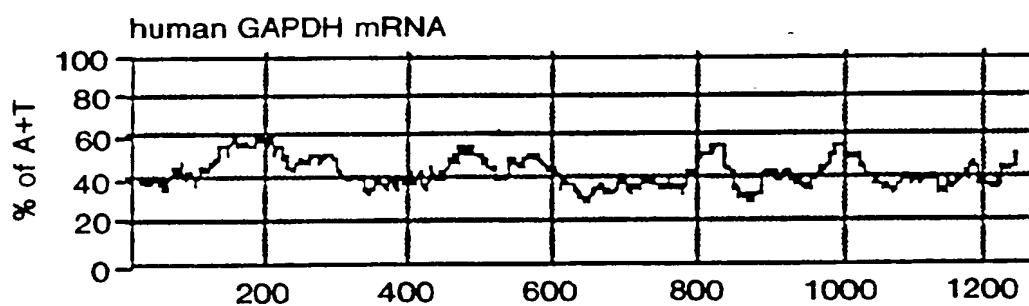


FIG. 11C

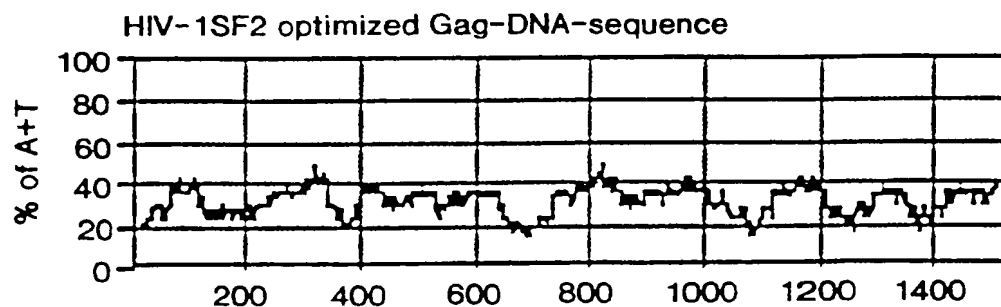


FIG. 11D

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native HIV-1SF2 gag-polymerase

ATGGTGCGAGAGCGCTCGGTATTAAGCGGGGAGAGATTAGATAAATGGAAAAAATTCGGTTAAGGCCAGGGGGAAG

Inact.1
 AAAAAATATAAGTTAAACATATATATGTTGGCAACGAGGAGCTAGAACGATTGCGATCAATCCTGGCCTGTTAGAA
 G G C C G C C

Inact.2
 ACATCAGAAGGCTGCAGACAAATATTGGGACAGCTACGCCATCCCTTCAGACAGGATCAGAAAGAACTTAGATCATT
 G G C C

Inact.3
 TATAATACAGTAGCAACCCCTCTATTGTGTACATCAAAAGGATAGATGTAAAAGACACCAAGGAAGCTTTAGAGAGATA
 C GC C C G

Inact.4
 GAGGAAGAGCAAAACAAAGTAAGAAAAAGGCACAGCAAGCCAGCAGCTGGCACAGGAAACAGCAGCCAGGTC
 GTCC G C G

AGCCAAAAATTACCCTATAGTGCAGAACCTACAGGGGCCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCA

TGGGTAAAAGTAGTAGAAGAAAGGCTTTCAGCCCCAGAAAGTAATACCCATGTTTTCAGCATTTATCAGAAAGGAGCCACC

Inact.5
 CCACAAAGATTAAACACCCATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAAATGTTAAAAGAGACTATCAAT
 G CC G G T G C

GAGGAAGCTCGAGAATGGGATAGAGTGCATCCAGTGCATGCAGGGCCCTATTGCACCGGCCAAATGAGAGAACCAGG

GGAACTGACATAGCAGGAACTACTAGTACCCCTTCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCCAGTA

Inact.6
 GGAGAAATCTATAAAAGATGGATAATCCTGGGATTAAATAAATAGTAAGAAATGTATAGCCCTACCAGCATTCTGGAC
 G C G G G C G C G G

ATAAGACAGGACCAAGGAACCCCTTAGAGATTATGTAGACCGGTTCTATAAAACTCTAAGAGCCGGAACAAGCTTCA

FIG. 12A

SUBSTITUTE SHEET (RULE 26)

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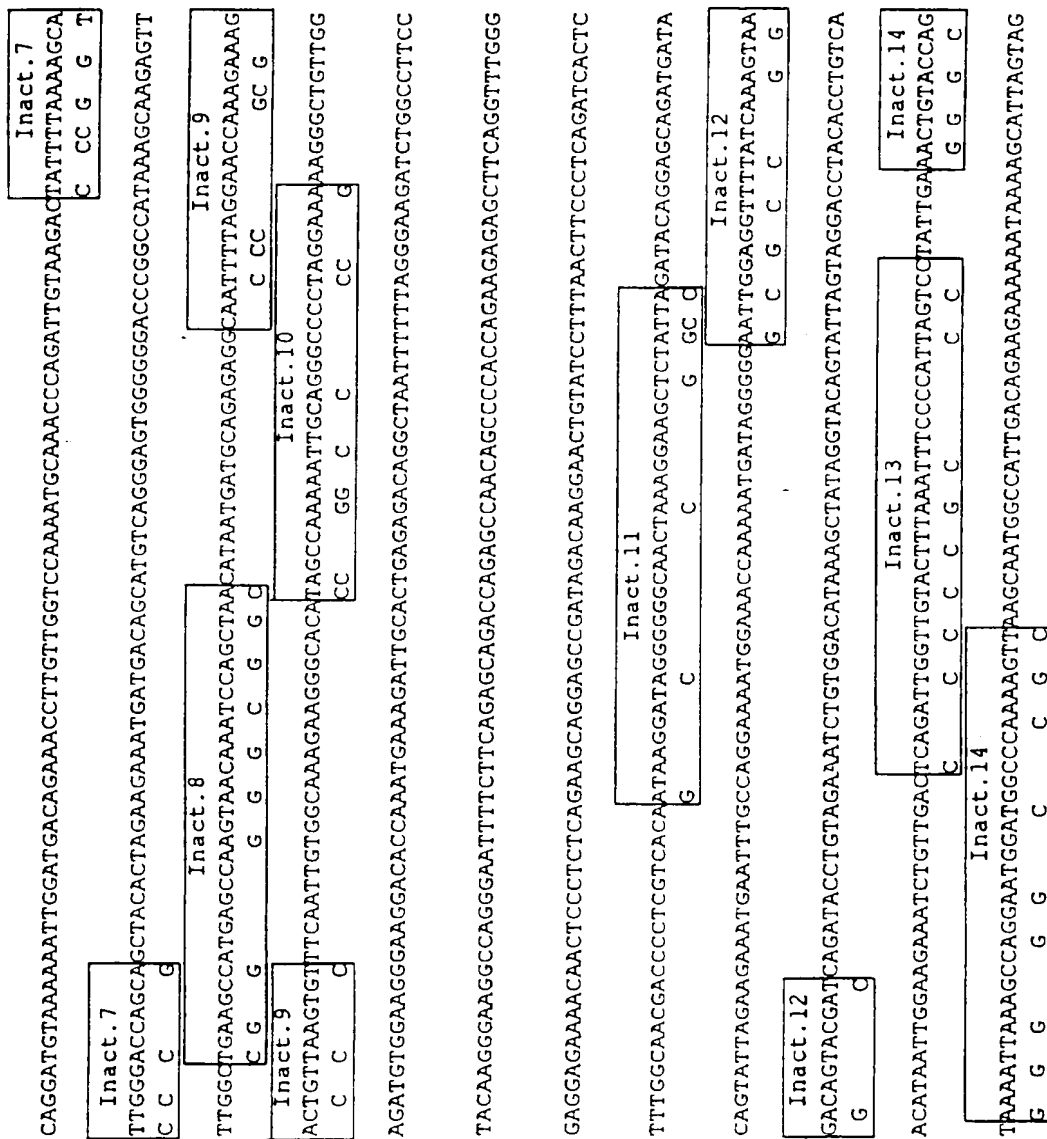


FIG. 12B

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AGATATGTACAGAAATGGAAAGGAAAGGAAATTTCAAAAATTTGGGCTGAAATCCATACATACTCCAGTATTTG
 CTATAAGAAAAGACAGTACTAAATGGAGAAAACATAGTAGATTTACAGAGAACTTAATAAAGAACTCAAGACTTCT
 GGAAGTTTCAGTTAGGAATACCAACCCCGCAGGTTTAAAAAGAAATAATCAGTAACAGTATTTGGATGGGTGATG
 CATACTTTTCAGTTCCCTTAGATAAAGACTTTAGAAAGTATCTACTGATTTACCATCTAGTATAAACAATGAGACAC
 CAGGGTTAGATATCAGTACRATGTCTGCCACAGGGATGGAAAGGATCACCGCAATATTTCCAAAGTAGCATGACAA
 AAATTTAGAGCCCTTTAGAAACAGAAATCCAGACATAGTTATCTATCAATACATGGATGATTTGTATGTAGGATCTG
 AACTTAGAATAGGGCAGCATAGAAACAAATAGAGAACTGAGACAGCATCTGTTGAGTGGGATTTACCAACACCG
 ACAAAAACATCAGAAAGAACCTCCATTCCTTTGGATGGGTATGAACCTGAGGAAATTTGAATTTGGGCAAGTCAGA
 TAATGCTGCCAGAAAAGACAGCTGGACTGTCAATGACATACAGAACTTACAGAACTTAACAGAACTAATACCACTAA
 TTTATGCAGGGATTAAGGAGTATGTAACTCTTAGAGGAAACCAAGCACTAACAGAACTAATACCACTAA
 CAGAAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTTAAAGAAACCAAGCACTAACAGAACTAATACCACTAA
 CAAAGACTTAGTAGCAAGAAATACAGAAAGCAGGGCAAGGCCAATGGACATCAAAATTTATCAAGAGGCCATTTAAAA
 ATCTGAAAACAGGAAGTATGCAAGGATGAGGGTCCCACTAATGATGTAAAACAGTTAAACAGAGGCGAGTGCAAA
 AAGTATCCACAGAAAGCATAGTAATCTGCTGATTTCTGAGTGGAGTTTGTCAATACCCCTCCCTTAGTGAATTTAT
 CATGGTGGATGGAGTATTTGGCAAGTACCTGGATTCCTGAGTGGAGTTTCTATGTAGTGGGCGAGCTAATAGGAGACTAAAT
 GGTACCAAGTTAGAGAAAGAACCCATAGTAGGAGCAGAAAATTTCTATGTAGTGGGCGAGCTAATAGGAGACTG
 TAGGAAAACAGGATATGTTACTGACAGAGGAAGACAAAAGTTTGTCTCCATAGCTGACACAAATCAGAAAGACTG
 AATTACAGCAATTCATCTAGCTTTGCAGGATTCGGGATTTAGAGTAACTAAGTAACTAGTAACAGACTCACAATATGCATTAG
 GAATCATTCAGCACAACCCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAAATAATAGAGCAGTTAATAAAGGAAA
 AGGTCTACCTGGCATGGGTACCAACACACAAAGGAATTTGGAGGAAATGAAACAAAGTAAATAGTAAATTTAGTCTGGAA
 TCAGGAAAGTACTATTTTGAATGGAATAGATAAGGCCCAAGAAATAGTAGCCAGCTGTGATAAATGTACGTAAAGGAG
 TGGCTAGTATTTAACTGCCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTAGTCACTACATCTAGAAAGGAAATTTATCC
 AAGCCATGCATGGACAAGTAGACTGTAGTCCAGGAATATGGCACTAGATTTGACACATCTAGAGGCAAGCAATAT
 TGCTAGCAGTTTCATGTAGCCAGTGGATATATAGAAAGCAAGTATTTCCAGCAGACAGAGGCGGCAAGCAATAT
 TTCTCTTAAATTTAGCAGGAAGATGGCCAGTAAACAAATACATACAGCAATGGCAGCAATTTCCAGTACTACCGG
 TTAAGGCCCTGTTGGTGGCAGGGATCAAGAGGAATTTGGCATTCCTTACATCCCAAGTCCCAAGTCAAGGAGTAG
 AATCTATGAATATGAATTAAGGAAATTTAGGACAGGTAGAGATCAGGCTGAACACCTTAAGACAGCAGTACAAA
 TGGCAGTATTCATCCACAATTTTAAAGAAAAGGGGGATTTGGGGATACAGTGCAGGGGAAAGATAGTAGACATAA
 TAGCAACAGACATACAACTAAAGAACTACAAAGCAATTTACAAAATTTCAAAATTTTCGGGTTTATTACAGGGACA
 ACAAGATCCCTTTGGAAAGGACCCAGCAAGCTTCTCTGAAAGGTGAAGGGCAGTAGTAATACAGATPAATAGTG
 ACATAAAGTAGTGCCCAAGAAAGAAAGCAAAAATCATTAGGGATTTATGGAAACAGATGGCAGGTGATGATTTGTGTGG
 CAAGTAGACAGGATGAGGATTAG

FIG. 12C

SUBSTITUTE SHEET (RULE 26)

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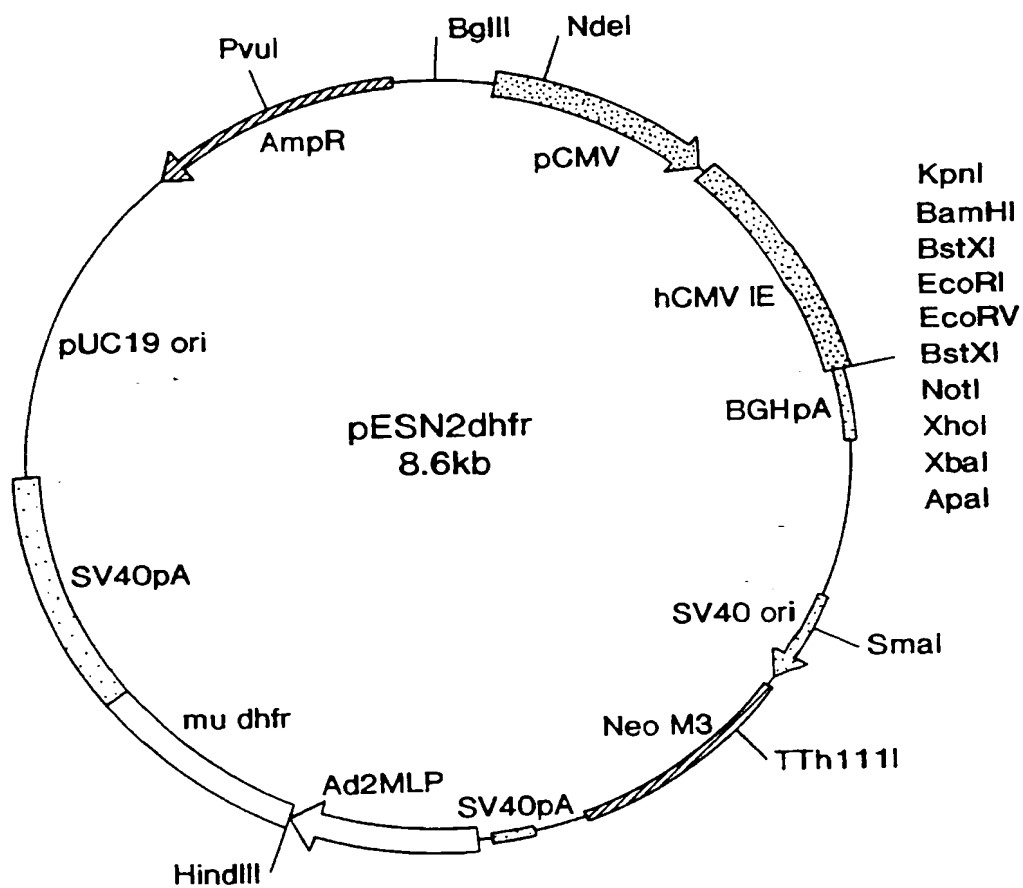


FIG. 13A

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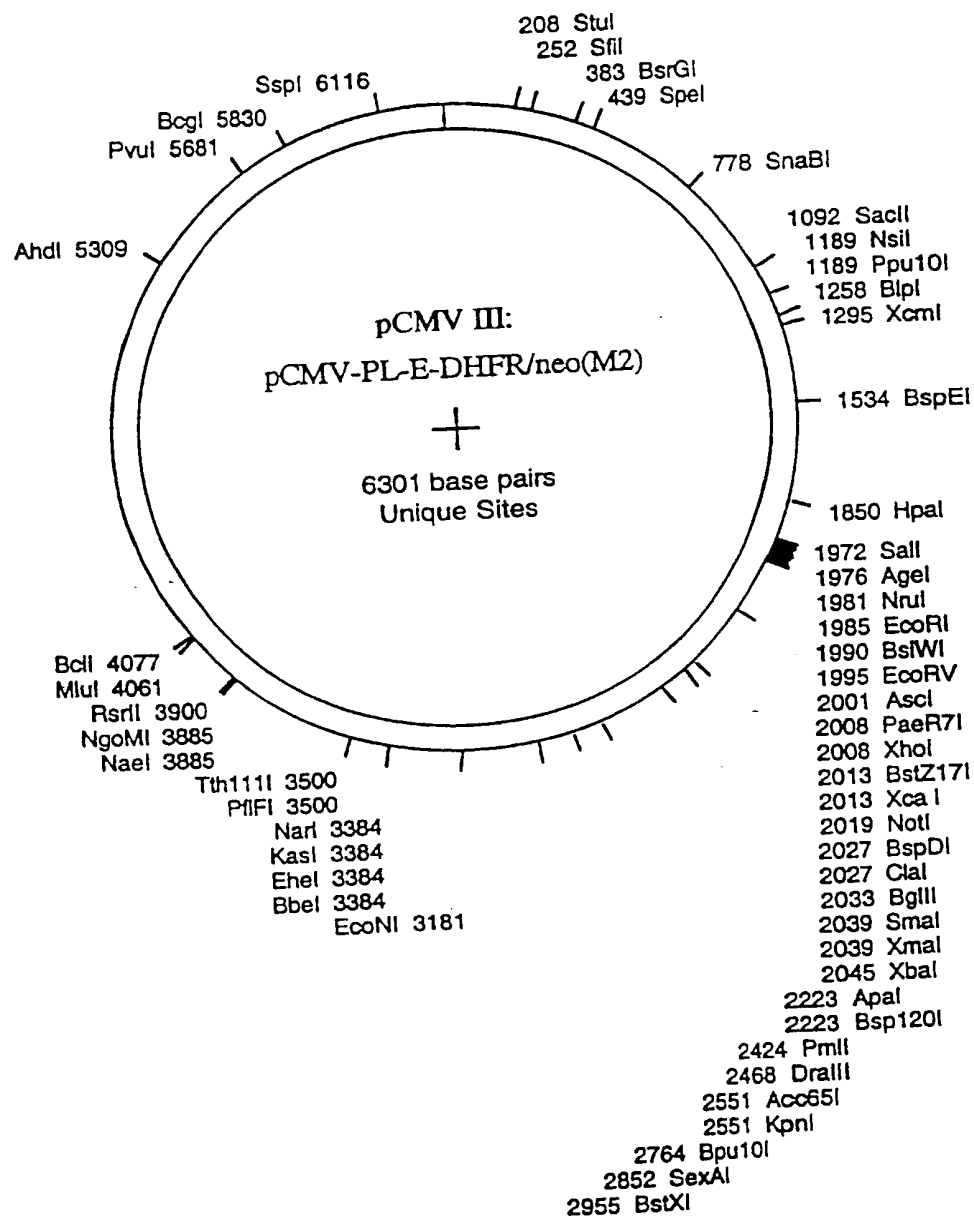


FIG. 13B

SUBSTITUTE SHEET (RULE 26)

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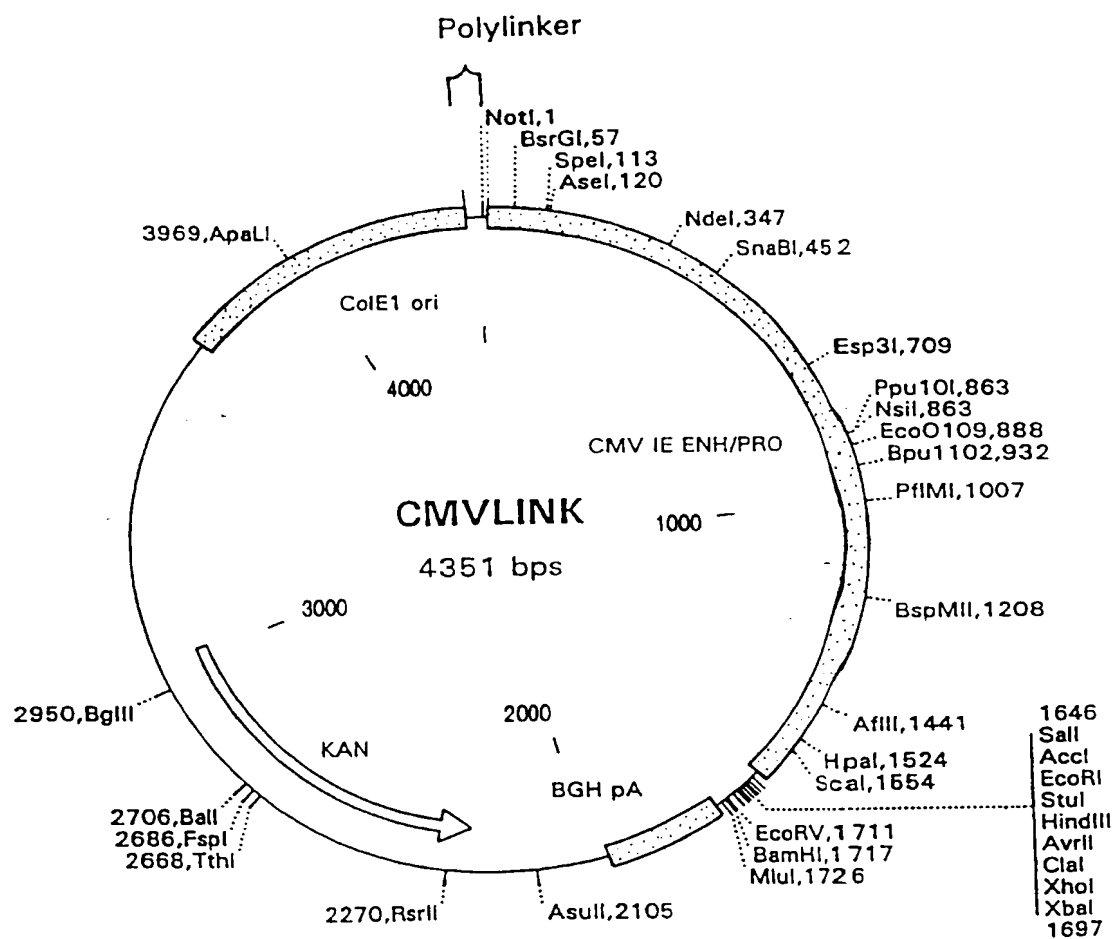


FIG. 14

SUBSTITUTE SHEET (RULE 26)

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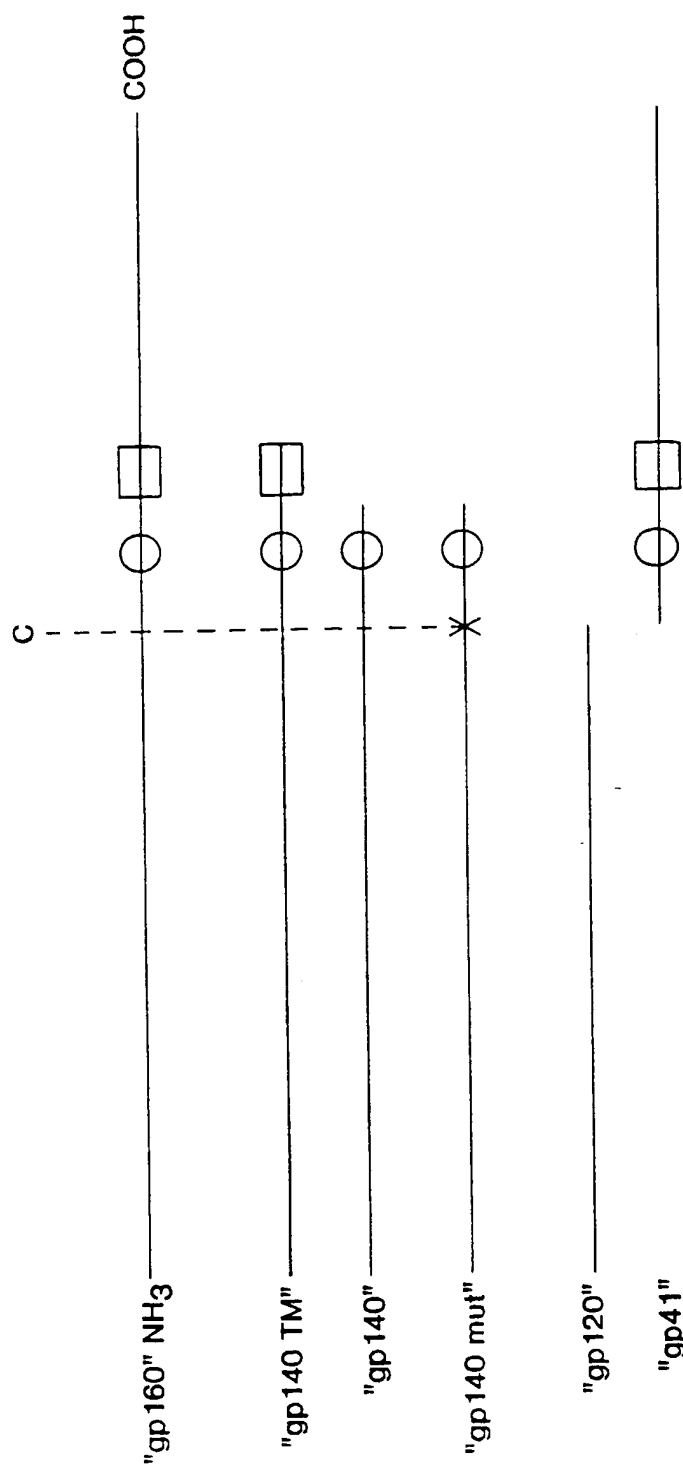


FIG. 15

SUBSTITUTE SHEET (RULE 26)

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gp120wtSF162

GTAGAAAAATTGTGGTCAAGTCTATTATGGGTACCTGTGTGGAAAGCAACCACCCTCTATTTT
GTGCATCAGATGCTAAAGCCTATGACACAGAGGTACATAATGCTGGGCCACACATGCCTGTGTACCCAC
AGACCTAACCCACAAGAAATAGTATTGGAAATGTGACAGAAAAATTTTAAATGTGGAAAAATAACATG
GTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAGTCTAAAGCCATGTGTAAAGTTAACCC
CACTCTGTGTTACTCTACATTGCACCTAATTTGAAGAAATGCTACTAATACCAAGAGTAGTAATTGGAAAGA
GATGGACAGAGGAGAAATAAAAAATTTGCTCTTTCAAGGTCAACCAAGCATAAGAAATAAGATGCAGAAA
GAATATGCACCTTTTATAAACTTGATGTAGTACCATAATAGATAATGATAATACAAAGCTATAAAATTGATAA
ATTGTAAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAACCAATTTCCCATACATTATTG
TGCCCGGCTGGTTTGGGATTCTAAAGTGTAAATGATAAGAAAGTTCAATGGATCAGGACCATGTACAAAT
GTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTGTCAACTCAAATTGCTGTTAAATGGCAGTC
TAGCAGAAAGAGGGGTAGTAATTAGATCTGAAAAATTTTCACAGACAAATGCTAAAACTATAATAGTACAGCT
GAAGGAATCTGTAGAAATTAATTGTACAAGACCTAACATAATACAAGAAAAAGTATACTATAGGACCG
GGGAGAGCATTTTATGCAACAGGAGACATAATAGGAGATATAAGACAAGCACATTTGTAACATTAGTGGAG
AAAAATGGAATAACACTTTAAAAACAGATAGTTACAAAATTACAAGCACAAATTTGGGAATAAAACAATAGT
CTTTAAGCAATCCTCAGGAGGGGACCCAGAAAATTGTAATGCACAGTTTAAATTGTGGAGGGAATTTTTC
TACTGTAATTCAACACAGCTTTTAAATAGTACTTGGATAATACTATAGGGCCAAATAACACTAATGGAA
CTATCACACTCCCATGCAGAAATAAAACAAATTATAAACAGGTGGCAGGAAGTAGGAAAAAGCAATGTATGC
CCCTCCCATCAGAGGACAAATTAGATGCTCATCAATATTACAGGACTGCTATTAAACAAGAGATGGTGGT
AAAGAGATCAGTAACACCCCGAGATCTTCAGACCTGGAGGTGGAGATATGAGGGACAAATTGGGAGAAGTG
AATTATATAAATATAAAGTAGTAAAAAATTGAGCCATTAGGAGTAGCACCCCAAGGCAAGAGAGAAGAGT
GGTGCAGAGAGAAAAAAGA

FIG. 16

(SEQ ID NO:30)

SUBSTITUTE SHEET (RULE 26)

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gp140wtSF162

GTAGAAAAATTGTGGGTACAGTCTATTATGGGGTACCTGTGTGGAAGAAGCAACCACCACCTCTATTTT
GTGCATCAGATGCTAAAGCCTATGACACAGAGGTACATAATGTCTGGGCCACACATGCTGTGTACCCAC
AGACCCCTAACCCACAAGAAATAGTATTGGAAAATGTGACAGAAAAATTTTAAACATGTGGAAAAATAACATG
GTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGTCTAAAGCCATGTGTAAAGTTAAACCC
CACTCTGTGTACTCTACATTGCACCTAAATTTGAAGATGCTACTATAATCCAAAGAGTAGTAATTTGGAAAA
GATGGACAGAGGAGAAATAAAAAATTTGCTCTTTCAAGGTCAACCAAGTCAATAGATAATACAAGCTATAAAATTGATAA
GAATATGCACCTTTTATAAACTTGATGTAGTACCAATAGATAATAGATAATACAAGCTATAAAATTGATAA
ATTGTAAACACCTCAGTCATTACACAGGCCCTGTCCAAAGGTAFCCTTTGAACCAATTTCCCATACATTATTG
TGCCCGGCTGGTTTGGGATTTCTAAAGTGTAAATGATAAAGAAAGTTCAATGGATCAGGACCATGTACAAAT
GTCAGCACAGTAGAATGTACACATGGAATTAGGCCAGTAGTGTCAACTCAATTTGCTGTTAAATGGCAGTC
TAGCAGAAAGAGGGTAGTAATTAGATCTGAAAATTTTACAGACAAATGCTAAAAAGTATAAATAGTACAGCT
GAAGGAATCTGTAGAAATTAATTGTACAAGACCTAACAAATAACAGAAAAAGTATAAATAGTACAGCCG
GGGAGAGCAATTTATGCAACAGGAGACATAATAGGAGATAAAGCAAGCACATTTGTAACATTAGTGGAG
AAAAATGGAATAACACTTTAAACACAGATAGTTACAAAATTACAGCACAATTTGGGAATAAAACAATAGT
CTTTAAGCAATCCTCAGGAGGGGCCCAAGAAATTTGAATGACACAGTTTAAATTTGTGGAGGGAAATTTTC
TACTGTAAATTCACACAGCTTTTAAATAGTACTTGAATAAATACTATAGGCCAAATAACACTAATGGAA
CTATCACACTCCCATGCAGAAATAAACAAATTAATAACAGGTGGCAGGAAGTAGGAAAAAGCAATGTATGC
CCCTCCCATCAGAGGACAAATTAGATGCTCATCAATAATTACAGGACTGCTATTAAACAAGAGATGGTGGT
AAAGAGATCAGTAACACCAACCGAGATCTTCAGACCTGGAGGTGGAGATATGAGGACCAATTTGGAGAAAGT
AATTATATAATAAAGTAGTAAAAATTTAGCCATTTAGGAGTAGCACCACCAAGGCCAAAGAGAGAGT
GGTGAGAGAGAAAAAAGAGCAGTGACGCTAGGAGCTATGTTCTTGGGTTCTTGGGAGCAGCAGGAAGC
ACTATGGGCGCAGGTCACTGACGCTGACGGTACAGGCCAGACAAATTAATGCTGTTAGTGTGCAACAGC
AGAACAAATTTGCTGAGAGCTATTGAGGGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCA
GCTCCAGGCAAGAGTCTGCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGGGATTTGGGGTTGC
TCTGGAAAACTCATTTGCACCCTGCTGTGCTGCTGGAAATGCTAGTTGGAGTAATAAATCTCTGGATCAGA
TTTGGGAATAACATGACCTGGATGGAGTGGGAGAGAGAAATTTGACAAATTACACAACTTAATATACACCTT
AATTGAAGAAATCGCAGAACCAACAGAAAAAGAAATTAATGAATTTGATAAAGTGGGCAAGT
TTGTGGAATTTGGTTTGCATATCAAAAATGGCTGTGGTATATA

FIG. 17

(SEQ ID NO:31)

SUBSTITUTE SHEET (RULE 26)

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gp160wtSF162

GTAGAAAAATTGTGGGTACAGTCTATTATGGGGTACCTGTGTGGAAAGAAGCAACCACCACTCTATTTT
GTGCATCAGATGCTAAAGCCTATGACACAGAGGTACATAATGTCTGGGCCACACATGCCTGTGTACCCAC
AGACCCTAACCCACAAGAAATAGTATTGGAAAAATGTGACAGAAAAATTTTAACATGTGGAAAAATAACATG
GTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGTCTAAAGCCATGTGTAAAGTTAACCC
CACTCTGTGTACTCTACATTGCACTAATTTGAAGAATGCTACTAATACCAAGAGTAGTAATTGGAAAGA
GATGGACAGAGGAGAAATAAAAAATTGCTCTTTCAAGGTCACCACAAGCATAAGAAATAAGATGCAGAAA
GAATATGCACTTTTTTATAAACTTGATGTAGTACCAATAGATAATGATAATACAAGCTATAAATTGATAA
ATTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAACCAATTCCCATACATTATTG
TGCCCCGGCTGGTTTTGCGATTCTAAAAGTGTAATGATAAGAAGTTCAATGGATCAGGACCATGTACAAAT
GTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTGTCAACTCAATTGCTGTTAAATGGCAGTC
TAGCAGAAGAAGGGGTAGTAATTAGATCTGAAAAATTTACAGACAATGCTAAAACCTATAATAGTACAGCT
GAAGGAATCTGTAGAAATTAATTGTACAAGACCTAAACAATAATACAAGAAAAAGTATAACTATAGGACCG
GGGAGAGCATTTTATGCAACAGGAGACATAATAGGAGATATAAGACAAGCACATTGTAACATTAGTGGAG
AAAAATGGAATAACACTTTAAACAGATAGTTACAAAATTACAAGCACAAATTTGGGAATAAAACAATAGT
CTTTAAGCAATCCTCAGGAGGGGACCCAGAAATTGTAATGCACAGTTTTAATTGTGGAGGGGAATTTTTTC
TACTGTAATTC AACACAGCTTTTAAATAGTACTTGGAAATAATACTATAGGGCCAAATAACACTAATGGAA
CTATCACACTCCCATGCAGAATAAAACAAATTATAAACAGGTGGCAGGAAGTAGGAAAAGCAATGGATGCT
CCCTCCCATCAGAGGACAAATTAGATGCTCATCAAATATTACAGGACTGCTATTAACAAGAGATGGTGGT
AAAGAGATCAGTAACACCACCGAGATCTTCAGACCTGGAGGTGGAGATATGAGGGACAATTGGAGAAGTG
AATTATATAAATATAAAGTAGTAAAAATTGAGCCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGT
GGTGCAGAGAGAAAAAAGAGCAGTGACGCTAGGAGCTATGTTCTTGGGTTCTTGGGAGCAGCAGGAAGC
ACTATGGGCGCACGGTCACTGACGCTGACGGTACAGGCCAGACAATTATTGTCCTGGTATAGTGCAACAGC
AGAACAATTTGCTGAGAGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCA
GCTCCAGGCAAGAGTCTTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGGGATTGGGGTTGC
TCTGGAAAACCTATTTGCACCACTGCTGTGCCTTGAATGCTAGTTGGAGTAATAAATCTCTGGATCAGA
TTTGGAATAACATGACCTGGATGGAGTGGGAGAGAGAAATTGACAATTACACAACTTAATATACACCTT
AATTGAAGAATCGCAGAACCAACAAGAAAAGAAATGAACAAGAATTATTAGAATTGGATAAGTGGGCAAGT
TTGTGGAATTGGTTTGACATATCAAAATGGCTGTGGTATATAAAAAATATTCATAATGATAGTAGGAGGTT
TAGTAGGTTTAAAGGATAGTTTTTACTGTGCTTTCTATAGTGAATAGAGTTAGGCAGGGATACTCACCATT
ATCATTTTCAGACCCGCTTCCAGCCCCAAGGGGACCCGACAGGCCCCAAGGAATCGAAGAAGAAGGTGGA
GAGAGAGACAGAGACAGATCCAGTCCATTAGTGCATGGATTATTAGCACTCATCTGGGACGATCTACGGA
GCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTAATCTTGATTGCAGCGAGGATTGTGGAACCTCT
GGGACGCAGGGGGTGGGAAGCCCTCAAGTATTGGGGGAATCTCCTGCAGTATTGGATTTCAGGAACATAAG
AATAGTGTCTGTAGTTTGTGTTGATGCCATAGCTATAGCAGTAGCTGAGGGGACAGATAGGATTATAGAAG
TAGCACAAAGAATTGGTAGAGCTTTTCTCCACATACCTAGAAGAATAAGACAGGGCTTTGAAAGGGCTTT
GCTATAA

FIG. 18

(SEQ ID NO:32)

SUBSTITUTE SHEET (RULE 26)

gp120.modSF162

ggaatctgcaccatggatgcaatgaagagagggctctgctgtgtgctgtgtgctgtgtgagcagctc
tctcgttctgcaccagcctggagaagctgtgggtgacctgtactacggcgtgccctgttggaag
gaggccaccaccacctgtctgcgcagcgacgccaaaggcctacgacaccgaggtgcacaacgtg
tggccaccaccgctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaaactcaacatgtggaagaacaacatggtggagcagatgcagaggacatcatcagcctgtgg
gaccagagcctgaagcctcgctgaagctgacccccctgtgcgtgacctgcaccaacctg
aagaacgccaccaacaacagagcagcaactggaaggagatggaccgggagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgcccctgttctacaagctg
gacgtggtgccatcgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accagggcctgccccaggctgagcttcgagcccatccccatccactactgcgccccgcggcttc
gccatcctgaagtgcacgacaagaagtcaacggcagcgccccctgcaccaacgtgagcacctg
cagtgacccacggcatccgcccctggtgagcaccacgtgctgctgaacggcagcctggccgag
gaggggctggtgatccgcagcgagaaactcaacgacaacgcccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcaccggcccccaacaacacccgcaagagcatcaccatcggcccc
ggcgcgccttctacgcccgcgacatcatcggcgacatccgcaggccccactgcaacatcagc
ggcgagaagtggaacaacacccctgaagcagatcgtgaccaagctgcaggccccagctcggcaacaag
accatcgtgttcaagcagagcagcgggcgaccccgagatcgtgatgcacagcttcaactgcggc
ggcgagtcttctactgcaacagcaccagctgttcaacagcacctggaacaacaccatcggcccc
aacaacaccaaggcaccatcacccctgccctgccgatcaagcagatcatcaaccgtggcaggag
gtgggcaaggccatgtacgcccccccatccgggccagatccgctgcagcagcaacatcacggc
ctgctgctgacccgcgacggcgcaaggagatcagcaacaccacccagatcttccgccccggcggc
ggcgacatcgcgcacaactggcgacgagctgtacaagtacaagtggtgaagatcgagccccctg
ggcgtggcccccaaggccaagcccgctggtgcagcgcgagaagcgttaactcgag

FIG. 19
(SEQ ID NO:33)

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[illegible]

FIG. 21
(SEQ ID NO:35)

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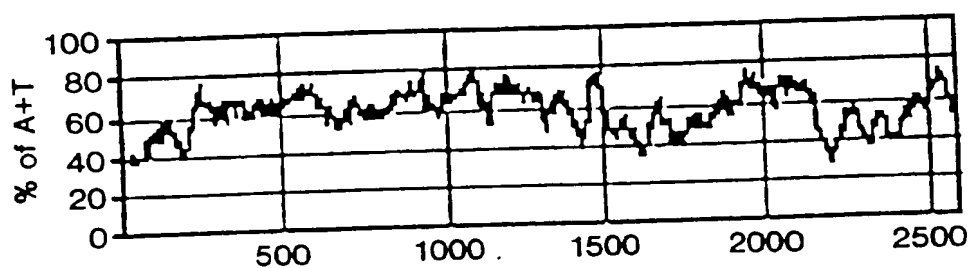


FIG. 22A

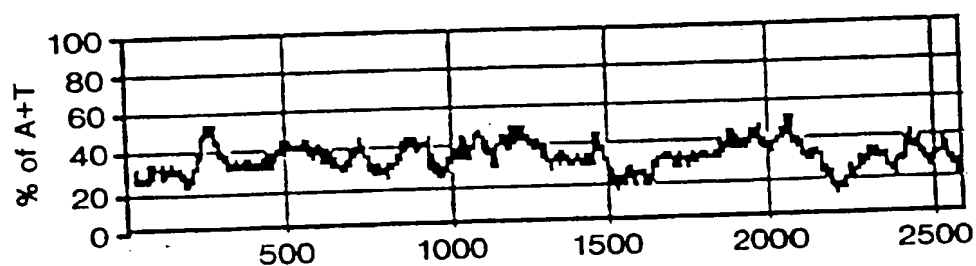


FIG. 22B

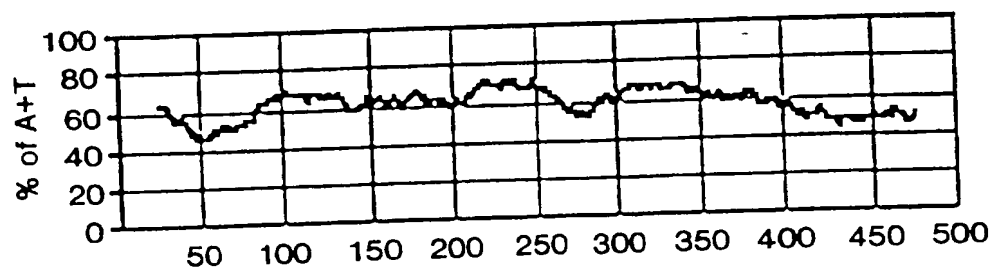


FIG. 22C

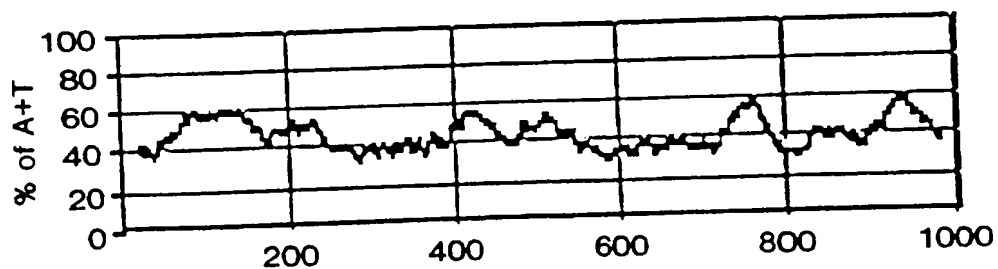


FIG. 22D

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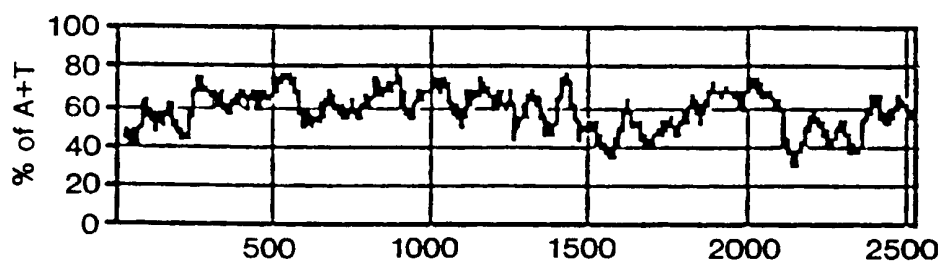


FIG. 22E

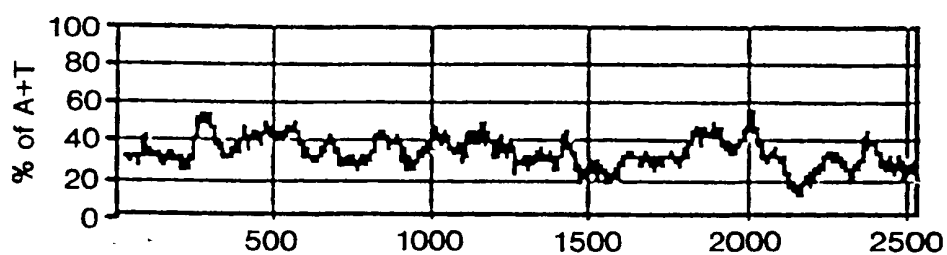


FIG. 22F

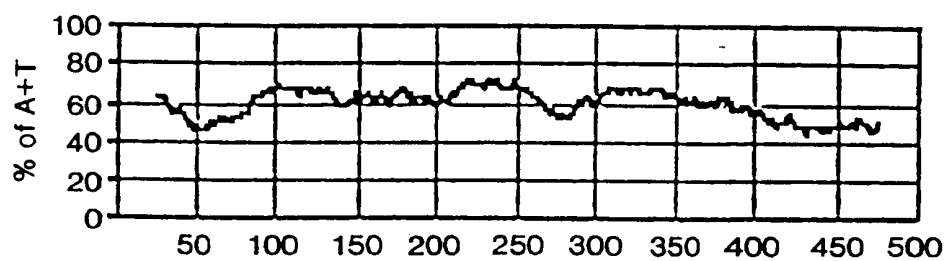


FIG. 22G

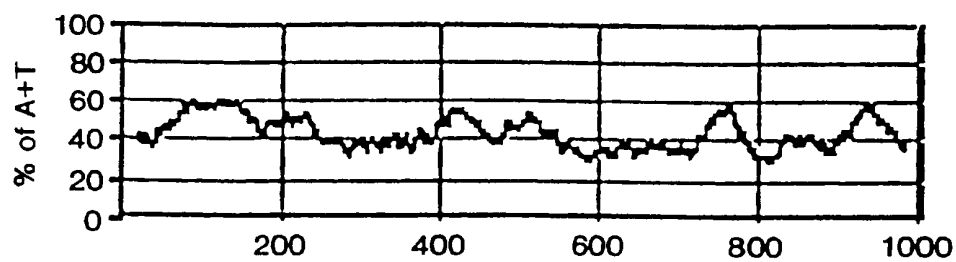


FIG. 22H

SUBSTITUTE SHEET (RULE 26)

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gp140.modSF162

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ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
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agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgccctgttctacaagctg
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gcatcctgaagtgcacgacaagaagttcaacggcgagcgccccctgcaccaacgtgagcaccgtg
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gagagcgtggagatcaactgcacccgcccccaacaacaacacccgcaagagcatcaccatcggtcccc
ggccgcgccttctacgccaccggcgacatcatcggtcgacatccgccaggccccactgcaacatcagc
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accatcgtgttcaagcagagcagcgggcgagcccccgagatcgtgatgcacagcttcaactgcggc
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aacaacaccaacggcaccatcacctgccccgcccgcagatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcggccagatccgctgcagcagcaacatcacccggc
ctgctgctgacccgcgacggcggaaggagatcagcaacaccaccagatcttccgccccggcggc
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ggcgtggccccccaccaaggccaagcgccgcgtgggtgcagcgcgagaagcgcgccgtgaccctgggc
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gcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggccccgcgtgctggcc
gtggagcgctacctgaaggaccagcagctgctggggcatctggggctgcagcggcaagctgatctgc
accaccgcccgtgcccgtgaacgccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgaggagcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtgg
aactggttcgacatcagcaagtggctgtggatcatctaactcgag

FIG. 23
(SEQ ID NO:36)

SUBSTITUTE SHEET (RULE 26)

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gp140.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggcaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgcactgcaccaacctg
aagaacgccaccaaacaccaagagcagcaactggaaggagatggaccgcccgcgagatcaagaactgc
agcttcaaggtgggcccggcaagctgatcaactgcaacaccagcgtgatcaccaggcctgcccc
aaggtgagcttcgagcccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagttcaacggcagcggccccctgcaccaacgtgagcaccgtgcagtgcaccacggc
atccgccccctgggtgagcaccagctgctgctgaacggcagcctggccgaggagggcgtgggtgatc
cgcagcggagaacttcaccgacaacgcccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcaccggcccccaacaacaacaccggcaagagcatcaccatcgcccccgccgcgccttctac
gccaccggcgacatcatcgggcagatccggccaggccactgcaacatcagcggcgagaagtggaac
aacacctgaagcagatcgtgaccaagctgcaggcccgagtccggcaacaagaccatcgtgttcaag
cagagcagcggcgaggcagccccgagatcgtgatgcacagcttcaactgcggcgggcgagtcttctctac
tgcaacagcaccagctgttcaacagcacctggaacaacacccatcgcccccaacaacaccaacggc
accatcaccttgccttgcgcgcatcaagcagatcatcaaccgctggcaggaggtgggcaaggccatg
tacgccccccccatccgcccgcagatccgctgcagcagcaacatcacccgctgctgctgacccgc
gacggcggcaaggagatcagcaacaccaccgagatcttccgccccggcgggcgacatgcgcgac
aactggcgagcagcgtgtacaagtacaaggtgggtgaagatcgagccccgggctggccccacc
aaggccaagcgcgcgtgggtgcagcgcgagaagcgcgcctgacccgggcccgcctgttcctgggc
ttcctgggcgcgcgcggcagcaccatgggcccgcagcctgaccccgaccgtgcaggccccgcag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccgagcagcactg
ctgcagctgaccgtgtggggcatcaagcagctgcaggcccgctgctggcctggagcgtacctg
aaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgcaccaccgcccgtgcc
tggaacgccagctggagcaacaagagcctggaccagatctggaacaacatgactggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtggaactgggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 24

(SEQ ID NO:37)

SUBSTITUTE SHEET (RULE 26)

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gp140.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcggtgcccggtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgcgtgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgcgtgaagctgacccccctgtgctggggcgccggcaactgccagacc
agcgtgatcaccagggcctgccccaaaggtgagcttcgagcccatcccatccactactgcgcccc
gcccggcttcgccatcctgaagtgcacgacaagaagttcaacggcagcgggccctgcaccaacgtg
agcaccgtgcagtgcacccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagc
ctggccgaggagggcggtggtgatccgcagcgagaacttcaccgacaacggccaagaccatcatcg
cagctgaaggagagcggtggagatcaactgcacccgcccccaacaacaccccgcaagagcatcacc
atcgcccccgccgccccttctacgcccacggcgacatcatcgggcagatccgccaggcccactgc
aacatcagcgggcgagaagtggacaacacccctgaagcagatcgtgaccaagctgcaggcccagttc
ggcaacaagaccatcgtgttcaagcagagcagcgggcgaccccgagatcgtgatgcacagcttc
aactgcggcgggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgcccccaacaacaccaacggcaccatcacccctgccctgccgcatcaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgccccccccatccgcggccagatccgctgcagcagcaac
atcacccggcctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttccgc
cccggcgggcgacatgcgcgacaactggcgagcgagctgtacaagtaacaggtggtgaagatc
gagccccctggcggtggccccccaccaaggccaagcgcccgctggtgcagcgcgagaagcgcgccgtg
accctggcgccatgttcttgggcttcttggcgcccgcccgagcaccatggcgccccgcagcctg
accctgaccgtgcaggcccccgagctgctgagcgccatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgacggctgtggggcatcaagcagctgcaggcccgc
gtgctggccgtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggaag
ctgatctgcaccacgcctgcccctggaacgccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacaccctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggccc
agcctgtggaactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 25

(SEQ ID NO:38)

SUBSTITUTE SHEET (RULE 26)

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gp140.mut.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcggtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggccaccacgcctgctgccccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgcactgcaccaacctg
aagaacgcccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgcccctgttctacaagctg
gacgtggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accagggcctgccccaaaggtgagcttcgagcccacccccatccactactgcgcccccgccggcttc
gccatcctgaagtgaacgacaagaagtccaacggcagcgccccctgcaccaacgtgagcaccgtg
cagtgcacccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgag
gaggcggtggtgatccgacgcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcacccgccccaaacaacacccgcaagagcatcaccatcgcccc
ggcgcgccttctacgccaccggcgacatcatcgccgacatccgcccaggcccaactgcaacatcagc
ggcgagaagtggaacaacacctgaagcagatcgtgaccaagctgcaggcccagttcggcaacaag
accatcgtgttcaagcagagcagcggcgccgagaccgagatcgtgatgcacagcttcaactgcccc
ggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccc
aacaacaccaacggcaccatcaccctgcccctgcccgcacagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcccgcagatccgctgcagcagcaacatcaccggc
ctgctgctgacccgcgacggcggaaggagatcagcaacaccaaccagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgagcagctgtacaagtacaagggtggtgaagatcgagccccctg
ggcgtggccccccaccaaggccaagcgccgctggtgcagcgcgagaagagcgccgtgacctgggc
gccatgttccctgggcttccctggcgccgcccgcagcaccatggcgccccgcagcctgacctgacc
gtgcaggccccgcagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgag
gcccagcagcacctgctgcagctgacctgtggggcatcaagcagctgcaggccccgcgtgctggcc
gtggagcgctacctgaaggaccagcagctgctgggcctctggggctgcagcggcaagctgatctgc
accaccgcccgtgccctggaacgccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgaggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtgg
aactggttcgacatcagcaagtggctgtggtacatctaaactcgag

FIG. 26

(SEQ ID NO:39)

SUBSTITUTE SHEET (RULE 26)

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gpl40.mut.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccaccctgttctgcccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgaggcgagatcaagaactgc
agcttcaaggtgggcgccggcaagctgatcaactgcaacaccagcgtgatcaccagggcctgcccc
aaggtgagcttcgagcccatcccatccactactgccccccgcccggcttcgccatcctgaagtgc
aacgacaagaagttcaacggcagcggccccctgcaccaacgtgagcacctgacgtgcacccacggc
atccgccccgtggtgagcaccacagctgctgctgaacggcagcctggccgaggaggcggtggtgatc
cgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcacccgcceccaacaacaacacccgcaagagcatcaccatcgcccccggccgcgccttctac
gccaccggcgacatcatcggcgacatccgcccaggcccactgcaacatcagcggcgagaagtggaaac
aacaccctgaagcagatcgtgaccaagctgcaggccccagttcggaacaagaccatcgtgttcaag
cagagcagcggcgggcgaccccgagatcgtgacacagcttcaactgcccggcgagttcttctac
tgcaacagcaccacagctgttcaacagcacctggaacaacaccatcgcccccacaacaccaacggc
accatcacccctgcccctgcccgcacatcaagcagatcatcaaccgctggcaggagggtgggcaaggccatg
tacgccccccccatccgcccgcagatccgctgcagcagcaacatcacccgcctgctgctgacccgc
gacggcgggcaaggagatcagcaacaccaccgagatcttccgccccggcgggcgacatgcgcgac
aactggcgcgagcagctgtacaagtacaagggtggtgaagatcgagcccctgggctggccccacc
aaggccaagcgccgctggtgacgcgcgagaagagcgccgtgacccctgggcgccatgttccctgggc
ttccctgggcccgcggcgacacatggggcgcccgacgctgacccctgacgctgcaggcccgccag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccagcagcacctg
ctgcagctgacccgtgtggggcatcaagcagctgcaggcccgcgtgctggcgcgtggagcgcctacctg
aaggaccagcagctgctggggcatctggggctgcagcggcaagctgatctgcaccaccgcccgtgccc
tggaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtggaactggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 27
(SEQ ID NO:40)

SUBSTITUTE SHEET (RULE 26)

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gp140.mut.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagaggggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccggtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctggtggcgccggcaactgccagacc
agcgtgatcaccacaggcctgcccgaaggtgagcttcgagcccatcccatccactactgcgcccc
gccggcttcgccatcctgaagtgcacgacaagaagttcaacggcagcgccctgcaccaacgtg
agcaccgtgcagtgcacccacggcatccgccccgtgggtgagcaccagctgctgctgaacggcagc
ctggccgaggagggcggtgatccgacgagagaacttcaccgacaacgccaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcacccgcccccaacaacaacacccgcaagagcatcacc
atcgggccccggcgcgcttctacgccaccggcgacatcatcgggcgacatccgccaggcccactgc
aacatcagcggcgagaagtggaaacaacacctgaagcagatcgtgaccaagctgcaggcccagttc
ggcaacaagaccatcgtgttcaagcagagcagcgggcgaccccgagatcgtgatgcacagcttc
aactgcggcgggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgggccccaacaacaccaacggcaccatcacctgcccgtgcgcgcatcaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgccccccccatccgcggccagatccgctgcagcagcaac
atcacccggcctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttcgc
cccggcgggcgacatgcgcgacaactggcgcgagcagctgtacaagtacaaggtggtgaagatc
gagccccctggcggtggcccccccaaggccaaagcgccgctggtgcagcgcgagaagagcgccgtg
accctggggcgccatgttcctgggcttcctggggcgccggcgagcaccatggggcgcccgagcctg
accctgaccgtgcaggcccgccagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccg
gtgctggccgtggagcgtacctgaaggaccagcagctgctgggcatctggggctgcagcggcaag
ctgatctgcaccaccgctgcccgtggaacgccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggccc
agcctgtggaactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 28

(SEQ ID NO:41)

SUBSTITUTE SHEET (RULE 26)

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gp140.mut7.modSF162

gaattcgccaccatggatgcaatgaagagaggggtctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgacctgtactacggcggtgcccggtgtggaag
gaggccaccaccacctgttctgcccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgacccccacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggtcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgcacctgttctacaagctg
gacgtggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
acccaggcctgccccaaaggtgagcttcgagcccatccccatccactactgcgcccccgccggcttc
gacctcctgaagtgcacgacaagaagtccaacggcagcgccccctgcaccaacgtgagcaccgtg
cagtgacccacggcatccgccccgtggtgagcaccagctgctgctgaaaggcagcctggccgag
gaggcggtggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcacccgccccacaacaacacccgcaagagcatcaccatcggtcccc
ggccgcgcttctacgccaccggcgacatcatcggtgacatccgcccaggccactgcaacatcagc
ggcgagaagtggaaacaacacctgaagcagatcgtgaccaagctgcaggccccagttcggcaacaag
accatcgtgttcaagcagagcagcggtggcgacccccgagatcgtgatgcacagcttcaactgcggc
ggcgagtcttctactgcaacagcaccagctgttcaacagcacctggaaacaacaccatcggtcccc
aacaacaccaacggcaccatcacctgccccgcatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcggtccagatccgctgcagcagcaacatcacgggc
ctgctgctgacccgcgacggcggtggaaggagatcagcaacaccaccagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgagcgagctgtacaagtacaagggtggtgaagatcgagccccctg
ggcggtggccccaccaggccatcagcagcgtggtgcagagcgagaagagcgccgtgacctgggc
gccatgttccctgggcttccctgggcgcccggcgagcaccatgggcgcccgcagcctgacctgacc
gtgcaggccccgcagctgctgagcggtcatcgtgcagcagcagaacaacctgctgcgcgcatcgag
gcccagcagcacctgctgcagctgacctgtggggcatcaagcagctgcaggccccgctgctggcc
gtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggtcaagctgatctgc
accaccgcccgtgccccggaacggcagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgggagcgcgagatcgacaactacaccaacctgatccacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggagaagtgggcccagcctgtgg
aactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 29

(SEQ ID NO:42)

SUBSTITUTE SHEET (RULE 26)

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gp140.mut7.modSF162.delv2

gaattcgccaccatggatgcaatgaagagaggggctctgctgtgtgctgctgctgtgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcgcgagatcaagaactgc
agcttcaaggtgggcgccggaagctgatcaactgcaacaccagcgtgatcaccagggcctgcccc
aaggtgagcttcgagcccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagttcaacggcagcggccccctgcaccaacgtgagcaccgtgcagtgcacccacggc
atccgccccgtggtagcaccacagctgctgctgaacggcagcctggccgaggagggcggtgatc
cgagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcacccgcccccaacaacaacaccccgcaagagcatcaccatcgcccccgccgccccttctac
gccaccggcgacatcatcggcgacatccgccaggccccactgcaacatcagcggcgagaagtggaaac
aacaccctgaagcagatcgtgaccaagctgcaggccccagttcggcaacaagaccatcgtgttcaag
cagagcagcggcgccgacccccgagatcgtgatgcacagcttcaactgcggcgggcgagttcttctac
tgcaacagcaccacagctgttcaacagcacctggaacaacaccatcgcccccaacaacaccaacggc
accatcacccctgcctgcccagatcaagcagatcatcaaccgctggcaggaggtgggcaaggccatg
tacgcccccccccatccgcccagatccgctgcagcagcaacatcaccggcctgctgctgacccgc
gacggcggaaggagatcagcaacaccacagagatcttcgccccggcgggcgacatgcgcgac
aactggcgagcagctgtacaagtacaaggtgctgaagatcgagccccctgggctggccccccacc
aaggccatcagcagcgtggtgcagagcagagaagagcgccgtgaccctgggcccctgttctgggc
tctctgggcccgcggcagcaccatgggcccgcagcctgaccctgaccgtgcaggccccgcag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccagcagcacctg
ctgcagctgaccgtgtggggcatcaagcagctgcaggccccgctgctggccgtggagcgtacctg
aaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgcaccaccgcccgtgccc
tggaaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtggaactgggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 30
(SEQ ID NO:43)

SUBSTITUTE SHEET (RULE 26)

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gp140.mut7.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcggtgcccgtgtggaag
gagggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggcaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctggtggcgccggcaactgccagacc
agcgtgatcaccaggeectgcccccaagggtgagcttcgagcccatcccatccactactgcgcccc
gcccgtctcgccatcctgaagtgaacgacaagaagttaacggcagcgccccctgcaccaacgtg
agcacogtgcagtgacccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagc
ctggccgaggagggcggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcacccgcccccaacaacaacaccgcaagagcatcacc
atcgcccccgccgcgcttctacgcccaccggcgacatcatcggcgacatccgcccaggcccactgc
aacatcagcggcgagaagtggaaacaacacctgaagcagatcgtgaccaagctgcaggcccagttc
ggcaacaagaccatcgtgttcaagcagagcagcgggcgagccccgagatcgtgatgcacagcttc
aactgcccggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgcccccaacaacaccaacggcaccatcacctgccccgcatcaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgccccccccatccgcccagatccgctgcagcagcaac
atcacccggcctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttcgc
ccccggcgccgacatgcgcgacaactggcgagcgagctgtacaagtacaagggtggtgaagatc
gagccccctggcggtggccccccaccaaggccatcagcagcgtggtgcagagcgagaagagcgccgtg
accctggcgcccatgttcctgggcttcctggcgccgcccggcagcaccatggcgcccgagcctg
accctgaccgtgcaggccccccagctgctgagcgccatcgtgcagcagcagaacaacctgctgcgc
gcatcagggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccgc
gtgctggccgtggagcgctacctgaaggaccagcagctgctggggcatctggggctgcagcggaag
ctgatctgcaccaccgcccgtgcccgggaacgcccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctcgagctggacaagtgggccc
agcctgtggaactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 31
(SEQ ID NO:44)

SUBSTITUTE SHEET (RULE 26)

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gpl40.mut8.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccaccctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgccccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgaccctgcaactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcgcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgcccctgttctacaagctg
gacgtgggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accagggcctgcccccaaggtgagcttgcagcccatccccatccactactgccccccgcccgttc
gccatcctgaagtgaacgacaagaagtccaacggcagcgccccctgcaccaacgtgagcacccgtg
cagtgcacccacggcatccgccccgtgggtgagcaccagctgctgctgaacggcagcctggccgag
gagggcgtgggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcacccgcccccaacaacacccgcaagagcatcaccatcgcccc
ggccgcgcccttctacgccaccggcgacatcatcgccgacatccgcccaggcccaactgcaacatcagc
ggcgagaagtggaaacaacaccctgaagcagatcgtgaccaagctgcaggcccagttcggaacaag
accatcgtgttcaagcagagcagcgggcgacccccgagatcgtgatgcacagcttcaactgcccc
ggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccc
aacaacaccaacggcaccatcacctgccccgccccgcatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgcccccccccatccgcgccagatccgctgcagcagcaacatcacggc
ctgctgctgaccccgacggcggaaggagatcagcaaacaccaccagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgagcagctgtacaagtacaaggctggaagatcgagcccctg
ggcgtggccccccaccatcgccatcagcagcgtgggtgcagagcgagaagagcgcctgaccctgggc
gccatgttccctgggcttccctggggcgccggcagcaccatggggcgcccgagcctgaccctgacc
gtgcaggccccccagctgctgagcggcatcgtgcagcagcagagaacaacctgctgcgccccatcgag
gcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggccccgctgctggcc
gtggagcgctacctgaaggaccagcagctgctggggcatctggggctgcagcggaagctgatctgc
accaccgcccgtgccctggaacgcccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtgg
aactgggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 32

(SEQ ID NO:45)

SUBSTITUTE SHEET (RULE 26)

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gp140.mut8.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcggtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgcaactgcaccaacctg
aagaacgcccaccaacaccaagagcagcaactggaaggagatggaccgcgcgagatcaagaactgc
agcttcaaggtgggcgccggcaagctgatcaactgcaacaccagcgtgatcaccaggcctgcccc
aaggtgagcttcgagccccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagttcaacggcagcgccctgcaaccaacgtgagcaccgtgcagtgcaccaacggc
atccgccccgtgggtgagcaccagctgctgctgaacggcagcctggccgaggaggcggtggtgatc
cgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcacccgcccccaacaacaacacccgcaagagcatcaccatcgcccccgccgcgccttctac
gccaccggcgacatcatcgggcgacatccgcccaggccactgcaacatcagcggcgagaagtggaaac
aacaccctgaagcagatcgtgaccaagctgcaggccagttcggaacaagaccatcgtgttcaag
cagagcagcgggcgggcgaccccgagatcgtgatgcacagcttcaactgcggcgggcgagtcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccccaacaacaccaacggc
accatcacccctgccctgcgcgatcaagcagatcatcaaccgctggcaggaggtgggcaaggccatg
tacgccccccccatccgcgccagatccgctgcagcagcaacatcaccggcctgctgctgaccgcg
gacggcgggcaaggagatcagcaacaccaccgagatcttccgccccggcgggcgacatgcgcgac
aactggcgcgagcagctgtacaagtacaaggtgggtgaagatcgagccccgggctggccccacc
atcgccatcagcagcgtggtgcagagcgagaagagcgccgtgacccctgggcgccatgttctgggc
ttctggggcgccgcccagcaccatgggcccgcagcctgacccctgaccgtgcaggccccgcag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccagcagcacctg
ctgcagctgaccgtgtggggcatcaagcagctgcaggccccgctgctggccgtggagcgtacctg
aaggaccagcagctgctgggcatctggggctgcagcggaagctgatctgcaccaccgcccgtgcc
tggaacgccagctggagcaacaagacctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtggaactggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 33

(SEQ ID NO:46)

SUBSTITUTE SHEET (RULE 26)

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gp140.mut8.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgcggtgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgcggtgaagctgacccccctgtgctgggcgccggcaactgccagacc
agcgtgatcaccagggcctgcccccaagggtgagcttcgagcccatccccatccactactgcgcccc
ggcgggttcgccatcctgaagtgcacacggaacttcaacggcagcggccccctgcaccaacgtg
agcacctgtcagtgacacccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagc
ctggccgaggagggcggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcacccgcccccaacaacaacaccccgcaagagcatcacc
atcgcccccgccgcgcttctacgccaccggcgacatcatcggcgacatccgccaggccccactgc
aacatcagcggcgagaagtgaacaacacccctgaagcagatcgtgaccaagctgcaggccccagttc
ggcaacaagaccatcgtgttcaagcagagcagcggcgggcgacccccgagatcgtgatgcacagcttc
aactgcggcgggcgagtcttcttactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgcccccaacaacaccaacggcaccatcaccttgccctgcccgcacatcaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgccccccccatccgcggccagatccgctgcagcagcaac
atcacccggcctgctgctgacccgcgacggcgggcaaggagatcagcaaacaccaccgagatcttccgc
cccggcgggcgacatgcgcgacaactggcgcgagcagctgtacaagtacaagggtggtgaagatc
gagccccctggcggtggccccaccatcgccatcagcagcgtggtgcagagcgagaagagcgccgtg
accctgggcgcatgttccctgggcttccctgggcgcccggcgagcaccatgggcgccccgcagcctg
accctgaccgtgcaggccccgacgtgctgagcggcatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggccccgc
gtgctggccgtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggcaag
ctgatctgcaccaccgcccgtgcccgggaacgccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcagagctgctggagctggacaagtgggccc
agcctgtggaactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 34

(SEQ ID NO:47)

SUBSTITUTE SHEET (RULE 26)

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gp160.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgaccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaagggtgaccaccagcatccgcaacaagatgcagaaggagtacgccctgttctacaagctg
gacgtggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
acccaggcctgcccgaagggtgagcttcgagcccatcccatccactactgcgcccccgccggttc
gccatcctgaagtgcacgacaagaagtccaacggcagcgccccctgcaccaacgtgagcacccgtg
cagtgacaccaacggcatccgccccgtgggtgagcaccagctgctgctgaacggcagcctggccgag
gagggcggtggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcacccgcccccaacaacaacaccgcaagagcatcaccatcgcccc
ggccgcgccttctacgccaccggcgacatcatcgggcgacatccgccaggccactgcaacatcagc
ggcgagaagtggaaacaacacctgaagcagatcgtgaccaagctgcaggcccgagttcggcaacaag
accatcgtgttcaagcagagcagcgggcgaccccgagatcgtgatgcacagcttcaactgcggc
ggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccc
aacaacaccaacggcaccatcacctgccccgcccgcacatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcccgcagatccgctgcagcagcaacatcacggc
ctgctgctgaccccgcgacggcgccaaggagatcagcaacaccaccgagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgagcgagctgtacaagtacaagggtggaagatcgagccccctg
ggcggtggcccccaacaggccaagcgccgctgggtgcagcgcgagaagcgcgccgtgaccctgggc
gccatgttccctgggcttccctgggcgccgcccgcagcaccatgggcccgcagcctgaccctgacc
gtgcaggccccgcagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgag
gcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggccccgctgctggcc
gtggagcgctacctgaaggaccagcagctgctggggcatctggggctgcagcggcaagctgatctgc
accaccgcccgtgcccctggaacgccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtgg
aactggttcgacatcagcaagtggctgtggtacatcaagatcttcatcatgatcgtgggcccctg
gtgggcccctgcgcacatcgtgttaccgtgctgagcatcgtgaaccgctgcgcccagggtacagcccc
ctgagcttccagacccgcttccccgcccccccgcgcccccgacgccccgagggcatcgaggaggag
ggcgggcagcgcgacccgcagcagccccctgggtgcacggcctgctggccctgatctgggac
gacctgcgcagcctgtgcctgttcagctaccaccgctgcgcgacctgatcctgatcgccgccccgc
atcgtggagctgctgggcccgcggctgggaggccctgaagtactggggcaacctgctgcagtac
tggatccaggagctgaagaacagcgccgtgagcctgttcgacgccatcgccatcgccgtggccgag
ggcaccgacccgcatcatcgaggtggcccagcgcatcgcccgccgcttccctgcacatcccccgccgc
atccgccagggttcgagcgccctgctgtaactcgag

FIG. 35

(SEQ ID NO:48)

SUBSTITUTE SHEET (RULE 26)

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gp160.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgaggcgagatcaagaactgc
agcttcaaggtgggcgccggcaagctgatcaactgcaacaccagcgtgatcaccaggcctgcccc
aaggtgagcttcgagccccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagttcaacggcgagcgccccctgcaccaacgtgagcaccgtgcagtgacccccggc
atccgccccgtggtgagcaccagctgctgctgaacggcgagcctggccgaggagggcggtggtgatc
cgcagcgagaacttcaacgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
gcccagccccgcccccaacaacaacaccgcaagagcatcaccatcgggcccgccgcccgttctac
aacaccctgaagcagatcgtgaccaagctgcaggccccagttcggaacaagaccatcgtgttcaag
cagagcagcgccggcgacccccgagatcgtgatgcacagcttcaactgcggcgaggagttcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacaccatcgggcccaacaacaccaacggc
accatcacccctgcccctgcccgcacagcagatcagcaacaccagatcctccgccccggcgccgacatgcgcgac
gacggcgccgaaggagatcagcaaacaccagagatcctccgccccggcgccgagacatgcgcgac
aactggcgagcgagctgtacaagtacaaggtggtgaagatcgagccccggcggtggccccacc
aaggccaagcgccgctggtgagcagcaccatggggcgcccgagcctgacccctgacccgtgcaggccccgag
ttcctggggcgccgcccggcagcaccatggggcgcccgagcctgacccctgacccgtgcaggccccgag
ctgctgagcgccatcgtgcagcagcagaacaacctgctgcgcgccatcgaggccccagcagcacctg
ctgcagctgacccgtgtggggcatcaagcagctgcaggccccgctgctggcggtggagcgtacctg
aaggaccagcagctgctggggcatctggggctgcagcgccaagctgatctgcaccaccgcccgtgccc
tggaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacaccctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtggaactggttcgacatc
agcaagtggctgtggtacatcaagatcttcatcatgatcgtggggcgccctggtgggcccgtgcgcatc
gtgttcaccgtgctgagcatcgtgaaccgctgcccagggctacagccccctgagcttccagacc
cgcttccccgcccccccgccgccccgagggccatcgaggaggagggcgccgagcgcgac
cgcgaccgcagcagccccctggtgcacggcctgctggccctgatctgggaagacctgcccagcctg
tgccgtgttcagctaccaccgctgcccagcctgatcctgatcgccgccccgcatcgtggagctgctg
ggcgccgcccgtgggagggccctgaagtactggggcaacctgctgcagtaactggatccaggagctg
aagaacagcgccgtgagcctgttcgacgccatcgccatcgccgtggccgagggcaccgaccgcatc
atcgaggtggccagcgcacggccgcccgttctgcacatcccccgccgcatccgcccagggttc
gagcgcccctgctgtaactcgag

FIG. 36

(SEQ ID NO:49)

SUBSTITUTE SHEET (RULE 26)

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gp160.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggcaccaccaccctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggcaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctggggcgccggcaactgccagacc
agcgtgatcaccaggcctgccccagggtgagcttcgagccccatccccatccactactgcgcccc
gccggcttcgccatcctgaagtgcacgacaagaagtcaacggcagcgccctgcaccaacgtg
agcaccgtgcagtgacccacggcatccgccccgtggtgagcaccagctgctgtgaacggcagc
ctggccgaggagggcggtgatccgcagcgagaacttcaccgacaacgcgaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcacccgcccccaacaacaacacccgcaagagcatcacc
atcgcccccgccgcgcttctacgccaccggcgacatcatcgccgacatccgccaggcccaactgc
aacatcagcggcgagaagtggaaacaacaccctgaagcagatcgtgaccaagctgcaggcccaagtcc
ggcaacaagaccatcgtgttcaagcagagcagcgccggcgacccccgagatcgtgatgcacagcttc
aactgcggcgggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgcccccaacaacaacagcgaccatcaccctgcccctgcgcgcatcaagcagatcatcaacgc
tggcaggaggtgggcaaggccatgtacgccccccccatccgcggccagatccgctgcagcagcaac
atcacgggctgctgctgacccgcgacggcgggcaaggagatcagcaacaccacccgagatcttcgc
ccccggcgccgagacatgcgcgacaactggcgcgagcagctgtacaagtacaagggtggtgaagatc
gagccccctggcgctggccccccaccaaggccaaagcgccgctggtgcagcgcgagaagcgcgccgtg
accctggcgccatgttccctgggcttccctgggcgccgcccggcagcaccatggcgccccgcagcctg
accctgaccgtgcaggcccgccagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccgagcagcaccctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccg
gtgctggccgtggagcgtacctgaaggaccagcagctgctgggcatctggggctgcagcggcaag
ctgatctgcaccaccgcccgtgcccggaaacgcccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggoc
agcctgtggaactggttcgacatcagcaagtggctgtggtacatcaagatcttcatcatgatcgtg
ggcgccctggtgggctgcgcatcgtgttcaccgtgctgagcatcgtgaaccgcgtgcccaggggc
tacagccccctgagcttccagaccgcttccccgcccccgccggccccgaccccccaggggcatc
gaggaggagggcgccgagcgcgacccgcgacccgagcagccccctggtgcaaggcctgctggccctg
atctgggacgacctgcgcagcctgtgacctgttcagctaccaccgcccgcgacctgatcctgatc
gccgccccgcatcgtggagctgctgggccccgcggctgggaggccctgaagtactggggcaacctg
ctgcagtactggatccaggagctgaagaacagcgccgtgagcctgttcgacgcccacgcccacgccc
gtggccgaggggaccgaccgcatcatcgaggtggcccagcgcacgcccgcgccccttctgcacatc
ccccgcgcgcatccgccagggttcgagcgcgcccctgctgtaactcgag

FIG. 37
(SEQ ID NO:50)

SUBSTITUTE SHEET (RULE 26)

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gp120wtUS4

ACAACAGTCTTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAG
CAACCACCACTCTGTTTTGTGCATCAGATGCTAAAGCATACAAAGCAGAGGC
ACATAACGTCTGGGCTACACATGCCTGTGTACCCACAGACCCCAACCCACAG
GAAGTAAATTTAACAAATGTGACAGAAAATTTAACATGTGGAAAAATAACA
TGGTGGAAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAA
GCCATGTGTAAAATTAACCCCACTCTGTGTTACTTTAAATTGTAAGTATAAGT
TGACAGGTAGTACTAATGGCACAATAAGTACTAGTGGCACTAATAGTACTAG
TGGCACTAATAGTACTAGTACTAATAGTACTGATAGTTGGGAAAAGATGCCA
GAAGGAGAAATAAAAACTGCTCTTTCAATATCACCACAAGTGTAAGAGATA
AAGTGCAGAAAGAATATTCTCTCTTCTATAAACTTGATGTAGTACCAATAGAT
AATGATAATGCTAGCTATAGATTGATAAATTGTAATACCTCAGTCATTACACA
AGCCTGTCCAAAGGTATCTTTTGAACCAATCCCATAATTATTGTGCCCCGG
CTGGTTTTGCGATTCTAAAGTGTAAGATAAGAAGTTCAATGGAACAGGACC
ATGTAAAAATGTCAGCACAGTACAATGCACACATGGAATTAGACCAGTAGTA
TCAACTCAACTGCTGTAAATGGCAGTCTAGCAGAAGAAGAGATAGTACTTA
GATCTGAAAATTTACAGACAATGCTAAAACCATAATAGTACAGCTGAATGA
ATCTGTAGAAATTAATTGTATAAGACCCAACAATAATACAAGAAAAAGTATA
CATATAGGACCAGGGAGAGCATTATGCAACAGGTGATATAATAGGAGACA
TAAGACAAGCACATTGTAACATTAGTAAAGCAAACCTGGACTAACACTTTAGA
ACAGATAGTTGAAAAATTAAGAGAACAATTTGGGAATAATAAAACAATAATC
TTTAATTCATCCTCAGGAGGGGACCCAGAAATTGTATTTACAGTTTTAATTG
TGGAGGGGAATTTTTCTATTGTAATACATCAAACTATTTAATAGTACCTGGA
ATATTACTGAAGAGGTAAATAAGACTAAAGAAAATGACACTATCATACTCCC
ATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGGAAAAGCAAT
GTATGCCCCCTCCCATCAGAGGACAAATTAATGTTCATCAAATATTACAGGG
CTGCTATTAAGTAGAGATGGTGGTACTAACAATAATAGGACGAACGACACCG
AGACCTTCAGACCTGGGGGAGGAAACATGAAGGACAATTGGAGAAGTGAAT
TATATAAATATAAAGTAGTAAGAATTGAACCATTAGGAGTAGCACCCACCCA
GGCAAAGAGAAGAGTGGTGCAAAGAGAGAAAAGA

FIG. 38

(SEQ ID NO:51)

SUBSTITUTE SHEET (RULE 26)

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gp140wtUS4

ACAACAGTCTTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAG
CAACCACCACTCTGTTTTGTGCATCAGATGCTAAAGCATACAAAGCAGAGGC
ACATAACGTCTGGGCTACACATGCCTGTGTACCCACAGACCCCAACCCACAG
GAAGTAAATTTAACAAATGTGACAGAAAATTTTAACATGTGGAAAAATAACA
TGGTGGAAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAA
GCCATGTGTAAAATTAACCCCACTCTGTGTTACTTTAAATTGTAAGTACTGATAAGT
TGACAGGTAGTACTAATGGCACAATAAGTACTAGTGGCACTAATAGTACTAG
TGGCACTAATAGTACTAGTACTAATAGTACTGATAGTTGGGAAAAGATGCCA
GAAGGAGAAAATAAAAACTGCTCTTTCAATATCACCACAAGTGTAAGAGATA
AAGTGCAGAAAGAATATTCTCTCTTCTATAAACTTGATGTAGTACCAATAGAT
AATGATAATGCTAGCTATAGATTGATAAATTGTAATACCTCAGTCATTACACA
AGCCTGTCCAAAGGTATCTTTTGAACCAATTCCCATACATTATTGTGCCCCGG
CTGGTTTTTGCATTCTAAAGTGTAAGATAAGAAGTTCAATGGAACAGGACC
ATGTAAAAATGTCAGCACAGTACAATGCACACATGGAATTAGACCAGTAGTA
TCAACTCAACTGCTGTAAATGGCAGTCTAGCAGAAGAAGAGATAGTACTTA
GATCTGAAAATTTACAGACAATGCTAAAACCATAATAGTACAGCTGAATGA
ATCTGTAGAAATTAATTGTATAAGACCCAACAATAATACAAGAAAAAGTATA
CATATAGGACCAGGGAGAGCATTTTATGCAACAGGTGATATAATAGGAGACA
TAAGACAAGCACATTGTAACATTAGTAAAGCAAACCTGGACTAACACTTTAGA
ACAGATAGTTGAAAAATTAAGAGAACAAATTTGGGAATAATAAAACAATAATC
TTTAATTCATCCTCAGGAGGGGACCCAGAAATTGTATTTACAGTTTTAATTG
TGGAGGGGAATTTTCTATTGTAATACATCACAACCTATTTAATAGTACCTGGA
ATATTACTGAAGAGGTAAATAAGACTAAAGAAAATGACACTATCATACTCCC
ATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGGAAAAGCAAT
GTATGCCCCTCCCATCAGAGGACAAATTAAATGTTTCATCAAATATTACAGGG
CTGCTATTAAGTATAGAGATGGTGGTACTAACAATAATAGGACGAACGACACCG
AGACCTTCAGACCTGGGGGAGGAAACATGAAGGACAATTGGAGAAGTGAAT
TATATAAATATAAAGTAGTAAGAATTGAACCATTAGGAGTAGCACCCACCCA
GGCAAAGAGAAGAGTGGTGCAAAGAGAGAAAAGAGCAGTGGGACTAGGAG
CTTTGTTTCATTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTC
AGTGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAACAG
CAGAACAATTTGCTGAGAGCTATTGAGGCGCAACAGCATCTGTTGCAACTCA
CGGTCTGGGGCATCAAACAGCTCCAGGCAAGAATCCTGGCTGTGGAAAAGATA
CCTAAAGGATCAACAGCTCCTAGGGATTTGGGGTTGCTCTGGAAAACCTATTT
GCACCACTACTGTGCCTTGGAACCTAGTTGGAGTAATAAATCTCTGACTGAG
ATTTGGGATAATATGACCTGGATGGAGTGGGAAAGAGAAAATTGGCAATTATA
CAGGCTTAATATACAATTTAATTGAAATAGCACAAAACCAGCAAGAAAAGAA
TGAACAAGAATTATTGGAATTAGACAAGTGGGCAAGTTTGTGGAATTGGTTT
GATATAACAACTGGCTGTGGTATATA

FIG. 39

(SEQ ID NO:52)

SUBSTITUTE SHEET (RULE 26)

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gp160wtUS4

ACAACAGTCTTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAG
CAACCACCACTCTGTTTTGTGCATCAGATGCTAAAGCATACAAAGCAGAGGC
ACATAACGTCTGGGCTACACATGCCTGTGTACCCACAGACCCCAACCCACAG
GAAGTAAATTTAACAAATGTGACAGAAAATTTTAACATGTGGAAAAATAACA
TGGTGGAAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAA
GCCATGTGTAAAATTAACCCCACTCTGTGTTACTTTAAATTGTAAGT
TGACAGGTAGTACTAATGGCACAAATAGTACTAGTGGCACTAATAGTACTAG
TGGCACTAATAGTACTAGTACTAATAGTACTGATAGTTGGGAAAAGATGCCA
GAAGGAGAAATAAAAACTGCTCTTTCAATATCACCACAAGTGTAAGAGATA
AAGTGCAGAAAGAATATTCTCTCTTCTATAAACTTGATGTAGTACCAATAGAT
AATGATAATGCTAGCTATAGATTGATAAATTGTAATACCTCAGTCATTACACA
AGCCTGTCCAAAGGTATCTTTTGAACCAATTCCCATAATTATTGTGCCCCGG
CTGGTTTTGCGATTCTAAAGTGTAAGATAAGAAGTTCAATGGAACAGGACC
ATGTAAAAATGTCAGCACAGTACAATGCACACATGGAATTAGACCAGTAGTA
TCAACTCACTGCTGTAAATGGCAGTCTAGCAGAAGAAGAGATAGTACTTA
GATCTGAAAATTTACAGACAATGCTAAAACCAATAAGTACAGCTGAATGA
ATCTGTAGAAATTAATTGTATAAGACCCCAACAATAACAAGAAAAAGTATA
CATATAGGACCAGGGAGAGCATTATGCAACAGGTGATATAATAGGAGACA
TAAGACAAGCACATTGTAACATTAGTAAAGCAAACCTGGACTAACACTTTAGA
ACAGATAGTTGAAAAATTAAGAGAACTTTGGGAATAATAAAACAATAATC
TTTAATTCATCCTCAGGAGGGGACCCAGAAATTGTATTTACAGTTTTAATTG
TGGAGGGGAATTTTCTATTGTAATACATCACAATTTAATAGTACCTGGA
ATATTACTGAAGAGGTAAATAAGACTAAAGAAAATGACACTATCATACTCCC
ATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGGAAAAGCAAT
GTATGCCCTCCCATCAGAGGACAAATTAAATGTTTCATCAAATATTACAGGG
CTGCTATTAAGTAGAGATGGTGGTACTAACATAATAGGACGAACGACACCG
AGACCTTCAGACCTGGGGGAGGAAACATGAAGGACAATTGGAGAAGTGAAT
TATATAAATATAAAGTAGTAAGAATTGAACCATTAGGAGTAGCACCCACCCA
GGCAAAGAGAAGAGTGGTGCAAAGAGAGAAAAGAGCAGTGGGACTAGGAG
CTTTGTTTATTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTC
AGTGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAACAG
CAGAACAATTTGCTGAGAGCTATTGAGGCGCAACAGCATCTGTTGCAACTCA
CGGTCTGGGGCATCAAACAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA
CCTAAAGGATCAACAGCTCCTAGGGATTTGGGGTTGCTCTGGAAAACCTATTT
GCACCACTACTGTGCCTTGGAACTCTAGTTGGAGTAATAAATCTCTGACTGAG
ATTTGGGATAATATGACCTGGATGGAGTGGGAAAGAGAAATTGGCAATTATA
CAGGCTTAATATACAATTTAATTGAAATAGCACAAAACCAGCAAGAAAAGAA
TGAACAAGAATTATTGGAATTAGACAAGTGGGCAAGTTTGTGGAATTGGTTT
GATATAACAACTGGCTGTGGTATATAAGAATATTCATAATGATAGTAGGAG
GCTTGATAGGTTTAAAGAATAGTTTTTGTGCTGTTCTATAGTGAATAGAGTT
AGGCAGGGATACTACCAATATCATTGCAGACCCGCTCCCAGCTCAGAGGG

FIG. 40A

(SEQ ID NO:53)

SUBSTITUTE SHEET (RULE 26)

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GACCCGACAGGCCCGAAGGAATCGAAGAAGAAGGTGGAGAGAGAGACAGA
GACAGATCCAATCGATTAGTGCATGGATTATTGGCACTCATCTGGGACGATCT
GCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTG
TAGCGAGGATTGTGGAACCTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAGTA
TTGGTGGAATCTCCTGCAGTATTGGAGTCAGGAGCTAAAGAGTAGTGCTGTT
AGTTTGTTTAATGCCACAGCAATAGCAGTAGCTGAAGGGACAGATAGGATTA
TAGAAATAGTACAAAGAATTTTATAGAGCTGTAATTCACATACCTAGAAGAAT
AAGACAGGGCTTGGAGAGGGCTTTACTATAA

FIG. 40B
(SEQ ID NO:53)

SUBSTITUTE SHEET (RULE 26)

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gp120.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAAGTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCGAGCGGCAC
CAACAGCACCGCGGCACCAACAGCACCGACCAACAGCACCGACAGCTGGGAGAAGATG
CCCGAGGGCGAGATCAAGAACTGCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCAGGCCTGCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGCAAGTGCACCCACGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTCACCGACAACGCCAAGACCATCATCGTGCAAGTGAACGAGTCCGTGGAGATCA
ACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGGCGGCGGAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGCCCCCACCAGGCCAAGCGCCGC
GTGGTGCAAGCGGAGAAGCGCTAAGATATCGGATCCTCTAGA

FIG. 41

(SEQ ID NO:54)

SUBSTITUTE SHEET (RULE 26)

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gp120.mod.US4.del128-194

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCACAAACGTGTGGGCCACCCACGCCTGCGTGCCCAACGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGGGGGCAGGGAAGTGCAGAGACCAGCGTGATCACCCAGGC
CTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGC
ACCGTGCAGTGCACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGG
CAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACCGACAACGCCAAGA
CCATCATCGTGCACTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAAAC
ACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCAT
CGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCG
AGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCCGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTT
CTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGA
ACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAAC
ATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTG
CAGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCA
CCAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGC
GAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGGCCCCACCCAGGC
CAAGCGCCGCGTGGTGAGCGCGAGAAGCGCTAAGATATCGGATCCTCTAGA

FIG. 42

(SEQ ID NO:55)

SUBSTITUTE SHEET (RULE 26)

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gp140.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGACTACGGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGGTGAACC
TGACCAACGTGACCGAGAATTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCTGTGCGTG
ACCTGAACTGCACCGACAAGCTGACCGGCAGCACCACGGCACCAACAGCACCAGCGGCAC
CAACAGCACCAGCGGCACCAACAGCACCAGCACCACAGCACCAGCCTGCGCGACAAGGTGCA
CCCGAGGGCGAGATCAAGAACTGCAGCTTCAACATCACCACAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACAGCGTGATCACCAGGCCTGCCCAAGGTGAGCTTCGAGT
CCATCCCCATCCACTACTGCGCCCCCGCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGCCCCCTGCAAGAAGCTGAGCACCCTGCAAGTGACCCACGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTCACCGACAACGCCAAGACCATCATCGTGACGCTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAACAACACGCGTAAAGAGCATCCACATCGGCCCGGCCGCGCTTCT
ACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCCGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACAGCCAGCTGTTCAACAGCACCTGGAACATCACCAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCA TCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCCTGTGCTGACCCGCGACGGCGGCACCAACAACAACCGCAACCA
CGACACCGAGACCTTCCGCCCCGGCGGCGCAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGGCCCCACCCAGGCCAAGCGCCGC
GTGGTGACGCGGAGAAGCGCGCCGTGGGCGCTGGGCGCCCTGTTTCATCGGCTTCTGGGCGC
GCCGGGAGCACCATGGGCGCCGCTCCGTGACCCGTGACCGTGACGGCCCGCCAGCTGCTGAG
CGGCATCGTGACGAGCAGACAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGC
AGCTGACCGTGTGGGCGCATCAAGCAGCTGACGGCCCGCATCCTGGCCGTGGAGCGCTACCTG
AAGGACCAGCAGCTGTGGGCTGAGGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
GCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
TGGAGTGGGAGCGGAGATCGGCAACTACCCGGCCTGATCTACAACCTGATCGAGATCGCC
CAGAACCAGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGT
GGAAGTGGTTCGACATCACCACCTGGCTGTGGTACATCTAAGATATCGGATCCTCTAGA

FIG. 43

(SEQ ID NO:56)

SUBSTITUTE SHEET (RULE 26)

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gp140.mut.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCAACCGTGCTGTGGGTGACCGTGTAACGCGTGCCCGTG
TGGAAGGAGGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAATTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCGACGGCAC
CAACAGCACCGAGCGGACCAAGCAGCACCAGCACCAACAGCACCGACAGCTGGGAGAAGATG
CCCGAGGGCGGAGATCAAGAACTGCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCCAGGCCTGCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGCAAGTGACCCACGGCATCCGCCCC
GTGGTGAGCAGCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAATTACCGACAACGCCAAGACCATCATCGTGCAAGTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCGCGCGCCTTCT
ACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCAATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGGCCCCACCCAGGCCAAGCGCCGC
GTGGTGACGCGGAGAAGAGCGCCGTGGGCGCCTGTTTATCGGCTTCCTGGGCGCC
GCCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACGGCCCGCCAGCTGCTGAG
CGGCATCGTGACGAGCAGACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACTGCTGC
AGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGCTACCTG
AAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCAACACCGT
GCCCTGGAAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
TGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCC
CAGAACCGAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGT
GGAAGTGGTTCGACATCACCAACTGGCTGTGGTACATCTAAGATATCGGATCCTCTAGA

FIG. 44

(SEQ ID NO:57)

SUBSTITUTE SHEET (RULE 26)

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gp140.TM.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGGTGCCCGTG
TGGAAGGAGGCCACCACCAACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCCAGCGGCAC
CAACAGCACCCAGCGGCACCAACAGCACCCAGCACCAACAGCACCCGACAGCTGGGAGAAGATG
CCCGAGGGCGAGATCAAGAACTGCAGCTTCAACATCACCACAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCAGGCCTGCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGCAAGTGCAACCGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAATTCACCGACAACGCCAAGACCATCATCGTGAGCTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCGCGCCGCGCTTCT
ACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGGCGGCGGAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGCCCCCACCCAGGCCAAGCGCCGC
GTGGTGACGCGGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGTTTCATCGGCTTCCTGGGCGCC
GCCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACGGCCCGCCAGCTGCTGAG
CGGCATCGTGAGCAGCAGAAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGC
AGCTGACCGTGTTGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGCTACCTG
AAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGAAGCTGATCTGCACCACCACCGT
GCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
TGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCTGATCTACAACCTGATCGAGATCGCC
CAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGT
GGAACCTGGTTCGACATACCAACTGGCTGTGGTACATCCGCATCTTCATCATGATCGTGGGCG
GCCTGATCGGCCTGCGCATCGTGTTCCGCGTGCTGAGCATCGTGTAAGATATCGGATCCTCTA
GA

FIG. 45
(SEQ ID NO:58)

SUBSTITUTE SHEET (RULE 26)

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Gp140modUS4.DV1V2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGC
TGTGTGGAGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACC
GTGTAACGCGGTGCCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCG
CCAGCGACGCCAAGGCTTACAAGGCCAGGCCACAACGTGTGGGCCACCCA
CGCCTGCGTGCCACCGACCCCAACCCCAAGGAGGTGAACCTGACCAACGTG
ACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGGGCGCCGGCC
AGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
CGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGC
CCCTGCAAGAACGTGAGCACCGTGAGTGACCCACGGCATCCGCCCCGTGG
TGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCT
GCGCTCCGAGAACTTCAACGACAACGCCAAGACCATCATCGTGACGTGAAC
GAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGTAAGAGCA
TCCACATCGGCCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCGA
CATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTC
GAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCCGGCAACAACAAGACCATC
ATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCA
ACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCAC
CTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT
CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGGCAAG
GCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGACGAGCAATATTA
CCGGCCTGTCTGACCCGCGACGGCGGCCACCAACAACAACCGCACCAACGA
CACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGC
GAGCTGTACAAGTACAAGGTGGTGCATCGAGCCCCTGGGCGTGGCCCCCA
CCCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAGCGCGCCGTGGGCCTGG
GCGCCCTGTTTCATCGGCTTCCTGGGCGCCGCGGGAGCACCATGGGCGCCGC
CTCCGTGACCCTGACCGTGCAGGCCCCGCCAGCTGCTGAGCGGCATCGTGACG
CAGCAGAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGCAGC
TGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCG
CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTG
ATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGA
CCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCA
ACTACACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACCAGCAGGA
GAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAA
CTGGTTTCGACATCACCAACTGGCTGTGGTACATCTAAGATATCGGATCCTCTA
GA

FIG. 46

(SEQ ID NO:59)

SUBSTITUTE SHEET (RULE 26)

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Gp140modUS4.DV2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGC
TGTGTGGAGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACC
GTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCG
CCAGCGACGCCAAGGCTTACAAGGCCGAGGCCACAACGTGTGGGCCACCCA
CGCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACCTGACCAACGTG
ACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCC
CCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGG
CACCAACAGCACCGAGCGGCACCAACAGCACCGGCACCAACAGCACCG
CACCAACAGCACCGAGCTGGGAGAAGATGCCCCAGGGCGAGATCAAGAA
CTGCAGCTTCAACATCGGCGCCGGCCGCTGATCAACTGCAACACCGAGCTG
ATCACCCAGGCCTGCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACT
GCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGG
CACCGGCCCTGCAAGAACGTGAGCACCCTGCAAGTGCACCCACGGCATCCGC
CCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGA
TCGTGCTGCGCTCCGAGAACTTACCGACAACGCCAAGACCATCATCGTGCA
GCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGT
AAGAGCATCCACATCGGCCCGCCGCGCCTTCTACGCCACCGGCGACATCA
TCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCAAGGCCAACTGGACCAA
CACCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAA
GACCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCAC
AGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAA
CAGCACCTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACAC
CATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTG
GGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCA
ATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCAC
CAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTG
GCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTG
GCCCCACCCAGGCCAAGCGCCGCGTGGTGACGCGGAGAAGCGCGCCGTG
GGCCTGGGCGCCCTGTTTCATCGGCTTCCTGGGCGCCGCGGAGCACCATGG
GCGCCGCTCCGTGACCCTGACCGTGACGGCCCGCCAGCTGCTGAGCGGCAT
CGTGACAGCAGCAGAACAACTGCTGCGCGCCATCGAGGCCAGCAGCACCTG
CTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCG
TGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGG
CAAGCTGATCTGCACCACCGCTGCCCTGGAACAGCAGCTGGAGCAACAAG
AGCCTGACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAG
ATCGGCAACTACACCGCCTGATCTACAACCTGATCGAGATCGCCAGAACC
AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCC
TGTGGAAGTGGTTCGACATCACCAACTGGCTGTGGTACATCTAAGATATCGG
ATCCTCTAGA

FIG. 47

(SEQ ID NO:60)

SUBSTITUTE SHEET (RULE 26)

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Gp140modmutUS4.DV1V2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGC
TGTGTGGAGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACC
GTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCG
CCAGCGACGCCAAGGCTTACAAGGCCGAGGCCACAACGTGTGGGCCACCC
ACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACCTGACCAACGT
GACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGA
GGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGGGCGCCGGC
CAGGCCTGCCCCAAGGTGAGCTTCGAGGCCCATCCCCATCCACTACTGCGCCC
CCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCCG
CCCCTGCAAGAACGTGAGCACCGTGCACTGCACCCACGGCATCCGCCCCGTG
GTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGC
TGCGCTCCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGCAGCTGAA
CGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGTAAGAGC
ATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCG
ACATCCGCCAGGCCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCCT
CGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCAT
CATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCACAGCTTC
AACTGCGGCGGCGAGTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCA
CCTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCA
TCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGGCAA
GGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAATATT
ACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCAACG
ACACCGAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAACCTGGCGCA
GCGAGCTGTACAAGTACAAGGTGGTGCAGCATCGAGCCCCTGGGCGTGGCCCC
CACCCAGGCCAAGCGCCGCGTGGTGCAGCGGAGAAAGAGCGCCGTGGGCCT
GGGCGCCCTGTTTCATCGGCTTCCTGGGCGCCGCGGGAGCACCATGGGCGCC
GCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCTGAGCGGCATCGTGC
AGCAGCAGAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGCA
GCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAG
CGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGC
TGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCT
GACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGG
CAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCCAGAACCAGCAG
GAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGG
AACTGGTTCGACATACCAACTGGCTGTGGTACATCTAAGATATCGGATCCTC
TAGA

FIG. 48

(SEQ ID NO:61)

SUBSTITUTE SHEET (RULE 26)

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gp140.mod.US4.del128-194

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCCAACGTGTGGGCCACCCACGCTGCGTGCCACCGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGGGGGCAGGGAAGTGCAGAGACCAGCGTGATCACCCAGGC
CTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGC
ACCGTGCAAGTGACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGG
CAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAAGTTCACCGACAACGCCAAGA
CCATCATCGTGCAAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAAC
ACGCGTAAGAGCATCCACATCGGCCCCGCGCCTTCTACGCCACCGGCGACATCAT
CGGCGACATCCGCCAGGCCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCCTCG
AGCAGATCGTGGAAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGGCGGCGACCCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTT
CTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGA
ACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAAC
ATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTG
CAGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCA
CCAACGACACCGAGACCTTCCGCCCCGCGGCGGCAACATGAAGGACAAGTGGCGCAGC
GAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGCCCCCACCAGGC
CAAGCGCCGCGTGGTGACGCGGAGAAGCGCGCCGTGGGCGTGGCGCCCTGTTTCATCG
GCTTCCTGGGCGCCGCGGAGCACCATGGGCGCCGCTCCGTGACCCGTGACCGTGACG
GCCCCAGCAGCAGCTGCTGAGCGGCATCGTGACGAGCAGACAACCTGCTGCGCGCCATCGA
GGCCCAGCAGCACCTGCTGACGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCA
TCCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGC
GGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCT
GACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACA
CCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACCAGCAGGAGAAGAACGAGCAG
GAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAAGTGGTTTCGACATCACCAACTG
GCTGTGGTACATCTAAGATATCGGATCCTCTAGA

FIG. 49

(SEQ ID NO:62)

SUBSTITUTE SHEET (RULE 26)

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gp140.mut.mod.US4.del128-194

GAATTGCCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCCACAACGTGTGGGCCACCCACGCCTGCGTGCCCAACGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGGGGGCAGGGAACTGCGAGACCAGCGTGATCACCAGGC
CTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGC
ACCGTGCAAGTGACCCACGGCATCCGCCCCGTGGTGAGCACCAGCTGCTGCTGAACGG
CAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACGACAACGCCAAGA
CCATCATCGTGCAAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAAC
ACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCAT
CGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCCTCG
AGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGGCGGCGACCCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTT
CTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCAGGAGGTGA
ACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAAC
ATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTG
CAGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCA
CCAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGC
GAGCTGTACAAGTACAAGGTGCTGCGCATCGAGCCCCCTGGGCGTGCCCCCACCAGGC
CAAGCGCCGCGTGGTGACGCGGAGAGAGCGCCGTGGGCCTGGGCGCCCTGTTTCATCG
GCTTCCTGGGCGCCGCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACG
GCCCCGAGCTGCTGAGCGGCATCGTGACGAGCAGACAACCTGCTGCGCGCCATCGA
GGCCCAGCAGCACCTGCTGACGCTGACCGTGTGGGCATCAAGCAGCTGCAGGCCCGCA
TCCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGC
GGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCT
GACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACA
CCGGCCTGATCTACAACCTGATCGAGATCGCCAGAACCAGCAGGAGAAGAACGAGCAG
GAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTG
GCTGTGGTACATCTAAGATATCGGATCCTCTAGA

FIG. 50

(SEQ ID NO:63)

SUBSTITUTE SHEET (RULE 26)

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gp160.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTGCCCGTG
TGGAAGGAGGGCCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAAACAATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCAGCGGCAC
CAACAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACCAGCAGCTGGGAGAAGATG
CCCGAGGGCGAGATCAAGAAGCTGCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGCCCTGCAAGAAGCTGAGCACCCTGCAAGTGCAACCGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTCACCGACAACGCCAAGACCATCATCGTGAGCTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAAACACGCGTAAGAGCATCCACATCGGCCCGGCCGCGCCTTCT
ACGCCACCGGCGACATCGGCGACATCCGCCAGGCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCCTCGAGCAGATCGTGGAGAAAGCTGCGCGAGCAGTTGCGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGCGCGCACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTACTGCAACACCAGCCAGCTGTTCAACAGCAGCTGGAACATCACCAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCTGCCCTGCCGATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGCCTGCTGCTGACCCGCGACGCGGCCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGGCGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGGCATCGAGCCCTGGGCGTGGCCCCCAACAGGCCAAGCGCCGC
GTGGTGACGCGGAGAAGCGCGCCGTGGGCGCTGGGCGCCCTGTTTATCGGCTTCCTGGGCGC
GCCGGGAGCACCATGGGCGCCGCTCCGTGACCCCTGACCGTGACGGCCCGCCAGCTGCTGAG
CGGCATCGTGACGAGCAGACAACCTGCTGCGCGCCATCGAGGCCCGCCAGCTGCTGAG
AGCTGACCGTGTGGGCGATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGCTACCTG
AAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCACCACCGT
GCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
TGGAGTGGGAGCGCGAGATCGGCAACTACCCGGCCTGATCTACAACTGATCGAGATCGCC
CAGAACCAGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGT
GGAAGTGGTTCGACATCACCAACTGGCTGTGGTACATCCGCATCTTATCATGATCGTGGGCG
GCCTGATCGGCCTGCGCATCGTGTTCCCGTGCTGAGCATCGTGAACCGCGTGCGCCAGGGCT
ACAGCCCCATCAGCCTGCAGACCCGCTGCCCGCCAGCGCGGCCCGACCGCCCCGAGGGC
ATCGAGGAGAGGGGCGGCGAGCGGACCGCGACCGCAGCAACCGCCTGGTGCACGGCCTGCT
GGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCTGTTAGCTACCAACCGCCTGCGCGACCT
GCTGCTGATCGTGGCCCGCATCGTGGAGCTGCTGGGCCCGCGCGGCTGGGAGGCCCTGAAGT
ACTGGTGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTT
AACGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGATCGTGACGCGCAT
CTTCCGCGCCGTGATCCACATCCCCCGCGCATCCGCCAGGCCTGGA GCGCGCCCTGCTGTA
AGATATCGGATCCTCTAGA

FIG. 51

(SEQ ID NO:64)

SUBSTITUTE SHEET (RULE 26)

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gp160.modUS4.delV1

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGGGCGCCGGCGGCGAGATCAAGAACTGCAGCTTCAACAT
CACCACCAGCGTGCGCGACAAGGTGCAGAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGG
TGCCCATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCAACACCAGCGTGATCACCC
AGGCCTGCCCCAAGGTGAGCTTCGAGGCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGCACC
GTGCAGTGCACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTG
GCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTACCGACAACGCCAAGACCATCATCGT
GCAGCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACACAACACGCGTAAGAGCA
TCCACATCGGCCCCGGCGCGCCTTCTACGCCACCGGCGACATCATCGGCGACATCCGCCAGG
CCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCTCGAGCAGATCGTGGAGAAGCTG
CGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAACAGCAGCAGCGCGCGGACCCCGA
GATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTT
CAACAGCACCTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCATCC
TGCCCTGCCGCATCCGCCAGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGACCCGCGAC
CCCCCATCCCGCGGCCAGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGACCCGCGAC
GGCGGCACCAACAACAACCGCACCAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACAT
GAAGGACAACCTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGGCGATCGAGCCCCCTGGGCG
TGGCCCCCACCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCG
GCCCTGTTTCATCGGCTTCTGGGCGCCGCGGAGCACCATGGGCGCCGCTCCGTGACCCCTG
ACCGTGCAGGCCCCGCGAGCTGCTGAGCGGCATCGTGACGAGCAGCAACAACCTGCTGCGCGC
CATCGAGGCCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCC
GCATCCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGC
GGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGAC
CGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCC
TGATCTACAACCTGATCGAGATCGCCCAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTG
GAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTTCGACATCACCAACTGGCTGTGGTACATC
CGCATCTTCATCATGATCGTGGGCGGCTGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGC
ATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGCTGCCCGCCCAG
CGCGGCCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCA
GCAACCGCCTGGTGACGGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCTGT
TCAGCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAGCTGCTGGGCC
GCCGCGGCTGGGAGGCCCTGAAGTACTGGTGAACCTGCTGCAGTACTGGAGCCAGGAGCTG
AAGAGCAGCGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCG
CATCATCGAGATCGTGACGCGCATCTTCCGCGCCGTGATCCACATCCCCCGCGCATCCGCCA
GGCCTGGAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA

FIG. 52

(SEQ ID NO:65)

SUBSTITUTE SHEET (RULE 26)

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gp160.mod.US4.delV2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGG
TGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAATTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGACCTGAACTGCACCGACAAGCTGACCGGCAGCACCAA
CGGCACCAACAGCACCAGCGGCACCAACAGCACCAGCGGCACCAACAGCACCAGCACCA
ACAGCACCAGCAGCTGGGAGAAGATGCCCCGAGGGCGAGATCAAGAAGTGCAGCTTCAAC
ATCGGCGCCGCGCCCTGATCAACTGCAACACCAGCGTGATCACCAGGCCTGCCCCAA
GGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCGCGGCTTCGCCATCCTGA
AGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAAGCTGAGCACCCTGCGAG
TGCACCCACGGCATCCGCCCCGTGGTGAGCACCAGCTGCTGCTGAACGGCAGCCTGGC
CGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTACCGACAACGCCAAGACCATCATCG
TGCAGCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGTAAG
AGCATCCACATCGGCCCCGCGCGCCTTCTACGCCACCGGCACATCATCGGCGACAT
CCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAGATCG
TGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAACAGCAGCAGC
GGCGGCGACCCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTG
CAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGAACAAGACCA
AGGAGAACGACACCATCATCTGCCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG
GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAA
TATTACCGGCTGTGCTGACCCGCGACGGCGGCCACCAACAACAACCGCACCAACGACA
CCGAGACCTTCCGCCCCGCGGCGGCCAACAATGAAGGACAACCTGGCGCAGCGAGCTGTAC
AAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGGCCCCCAACCAGGCCAAGCGCCG
CGTGGTGACGCGGAGAAAGCGCGCCGTGGGCGCTGGGCGCCCTGTTTCATCGGCTTCCTGG
GCGCCGCGGGAGCACCATGGGCGCCGCTCCGTGACCCCTGACCGTGACGGCCCGCCAG
CTGCTGAGCGGCATCGTGACGAGCAGACAACCTGCTGCGCGCCATCGAGGCCCAGCA
GCACCTGCTGACGCTGACCGTGTGGGCGATCAAGCAGCTGACGGCCCGCATCCTGGCCG
TGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTG
ATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGAT
CTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGA
TCTACAACCTGATCGAGATCGCCCAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTG
GAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTTCGACATCACCAACTGGCTGTGGTA
CATCCGCATCTTCATCATGATCGTGGGCGGCTGATCGGCCTGCGCATCGTGTTTCGCCG
TGCTGAGCATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGC
CTGCCCCGCCAGCGCGGCCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCG
CGACCGCGACCGCAGCAACCGCCTGGTGACCGCCTGCTGGCCCTGATCTGGGACGACC
TGCGCAGCCTGTGCCTGTTTCAGCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGCC
CGCATCGTGAGCTGCTGGGCGCGCGCTGGGAGGCCCTGAAGTACTGGTGGAACCT
GCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTCAACGCCACCG
CCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGATCGTGACGCGCATCTTCCGC
GCCGTGATCCACATCCCCCGCCGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGA
TATCGGATCCTCTAGA

FIG. 53

(SEQ ID NO:66)

SUBSTITUTE SHEET (RULE 26)

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gp160.modUS4delV1/2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCAACCGTGCTGTGGGTGACCGTGTACTACGGCGTGCCCGTG
TGGAAGGAGGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGGAGAACTTCAACATGTGGAAGAAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGGGCGCCGCGCCAGGCCTGCCC
CAAGGTGAGCTTCGAGCCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAA
GTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGCAAGTGCA
CCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAG
GAGATCGTGCTGCGCTCCGAGAACTTACCGACAACGCCAAGACCATCATCGTGACGCTGAA
CGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAAACACGCGTAAGAGCATCCACATCG
GCCCCGCGCGCCTTCTACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCATCGA
ACATCAGCAAGGCCAACTGGACCAACACCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAG
TTCGGCAACAACAAGACCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTT
CCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCAC
CTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCC
GCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATC
CGCGGCCAGATCAAGTGACGAGCAATATTACCGCCTGCTGCTGACCCGCGACGGCGGCAC
CAACAACAACCGCACCAACGACACCGAGACCTTCCGCCCCGGCGGCGCAACATGAAGGACA
ACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGCCCCC
ACCCAGGCCAAGCGCCGCTGGTGACGCGGAGAAGCGCGCCGTGGGCTGGGCGCCCTGTT
CATCGGCTTCTGGGCGCCGCCGGGAGCACCATGGGCGCCGCTCCGTGACCTGACCGTGCA
GGCCCGCCAGCTGCTGAGCGGCATCGTGACGAGCAGAGAACAACCTGCTGCGCGCCATCGAGG
CCCAGCAGCACCTGCTGACGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTG
GCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCT
GATCTGCACCAACACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCT
GGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTAC
AACCTGATCGAGATCGCCCAGAACCCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGG
ACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGGCTGTGGTACATCCGCATCT
TCATCATGATCGTGGGCGGCCTGATCGGCCTGCGCATCGTGTTCCGCGTGCTGAGCATCGTGA
ACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAAGACCCGCTGCCCGCCCAGCGCGGC
CCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCAGCAACC
GCCTGGTGACCGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCTGTTAGCT
ACCACCGCCTGCGCGACCTGCTGCTGATCGTGCCCGCATCGTGGAGCTGCTGGGCGCGCGG
GCTGGGAGGCCCTGAAGTACTGGTGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGC
AGCGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCAT
GAGATCGTGACGCGCATCTTCCGCGCCGTGATCCACATCCCCCGCCGCATCCGCCAGGGCCTG
GAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA

FIG. 54

(SEQ ID NO:67)

SUBSTITUTE SHEET (RULE 26)

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gp160.modUS4 del 128-194

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGCTGCCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCTGCGTGCCACCGACCCCAACCCCAAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
GGGGCAGGGAACTGCGAGACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTTCGAGCC
CATCCCCATCCACTACTGCGCCCCCGCGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTT
CAACGGCACCCGGCCCTGCAAGAACGTGAGCACCCTGCGTGCAGTGACCCACGGCATCCGCCCCG
TGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCC
GAGAACTTCACCGACAACGCCAAGACCATCATCGTGCAGCTGAACGAGTCCGTGGAGATCAA
CTGCATCCGCCCCAACAAACACGCGTAAGAGCATCCACATCGGCCCGGCCGCGCTTCTA
CGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCACTGCAACATCAGCAAGGCCAACT
GGACCAACACCCCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACC
ATCATCTTCAACAGCAGCAGCGCGGCCGACCCCGAGATCGTGTTCACAGCTTCAACTGCGGC
GGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAG
GTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGATCCGCCAGATCATCAA
CATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCA
GCAGCAATATTACGGCCTGCTGCTGACCCGCGAGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGTA
GACACCGAGACCTTCCGCCCCGGCGGCGCAACATGAAGGACAACCTGGCGCAGCGAGCTGTA
CAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGCCCCCACCAGGCCAAGCGCCGCG
TGGTGACGCGGAGAGCGCGCCGCTGGGCGCTGGGCGCCCTGTTTCATCGGCTTCTGGGCGCCG
CCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACGGCCGCCAGCTGCTGAGC
GGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGCA
GCTGACCGTGTTGGGCGATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGCTACCTGA
CCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGAT
GGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCCC
AGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTG
GAACTGGTTTCGACATCAACCAACTGGCTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGG
CCTGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCATCGTGAACCGCGTGCGCCAGGGCTA
CAGCCCCATCAGCCTGCAGACCCGCTGCCCCGCCAGCGCGGCCCGGACCGCCCCGAGGGCA
TCGAGGAGGAGGGCGGCGAGCGCGACCGCAGCAACCGCCTGGTGACGGCCTGCTG
GCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCTGTTACGCTACCAACCGCCTGCGCGACCTG
CTGCTGATCGTGGCCCGCATCGTGGAGCTGCTGGGCGCGCGGCTGGGAGGCCCTGAAGTAC
TGGTGGAACTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTCAA
CGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCTT
CCGCGCCGTGATCCACATCCCCCGCCGATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGA
TATCGGATCCTCTAGA

FIG. 55

(SEQ ID NO:68)

SUBSTITUTE SHEET (RULE 26)

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Env_US4_C4wt

GACACTATCATACTCCCATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGG
AAAAGCAATGTATGCCCCCTCCCATCAGAGGACAAATTAAATGTTTCATCAAATATTACAG
GGCTGCTATTAAGTAGAGATGGTGGT

FIG. 56

(SEQ ID NO:69)

Env_SF162_C4wt

GGAACATATCACAATCCCATGCAGAATAAAACAAATTATAAACAGGTGGCAGGAAGTAGG
AAAAGCAATGTATGCCCCCTCCCATCAGAGGACAAATTAGATGCTCATCAAATATTACAG
GACTGCTATTAACAAGAGATGGTGGT

FIG. 57

(SEQ ID NO:70)

Env_US4_C4mod

GACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGG
CAAGGCCATGTACGCCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAACATCACCG
GCCTGCTGCTGACCCGCGACGGCGGC

FIG. 58

(SEQ ID NO:71)

Env_SF162_C4mod

GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCAGGAGGTGGG
CAAGGCCATGTACGCCCCCCCCCATCCGCGGCCAGATCCGCTGCAGCAGCAACATCACCG
GCCTGCTGCTGACCCGCGACGGCGGC

FIG. 59

(SEQ ID NO:72)

SUBSTITUTE SHEET (RULE 26)

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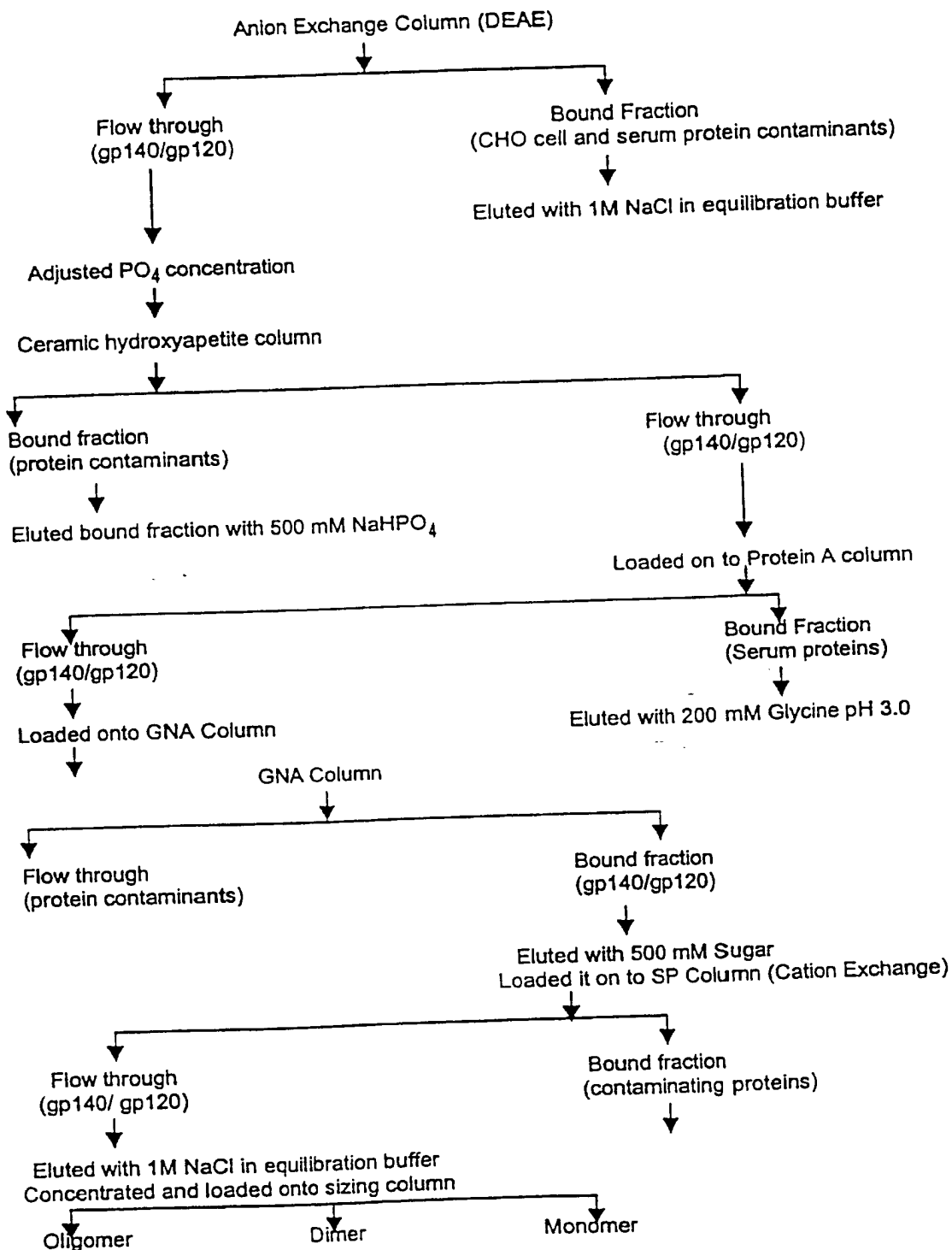


FIG. 60

SUBSTITUTE SHEET (RULE 26)

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gp160mod.us4.gag.modSF2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGA
GCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG
CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAG
GCCGAGGCCCAACCGTGTGGGCCACCCACGCCTGCGTGCCCAACGACCCCAACCCCCAG
GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG
CAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG
ACCCCCCTGTGCGTGACCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACC
AACAGCACCAGCGGCACCAACAGCACCAGCGGACCAACAGCACCAGCACCAACAGCACC
GACAGCTGGGAGAAGATGCCCCGAGGGCGAGATCAAGAAGTGCAGCTTCAACATCACCACC
AGCGTGCGCGACAAGGTGCAGAAGGATACAGCCTGTTCTACAAGCTGGACGTGGTGCCC
ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCAACACCAGCGTGATCACCAG
GCCTGCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCGGGCTTC
GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGCCCCCTGCAAGAAGCTGAGC
ACCGTGCAAGTGACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC
AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTACCGACAACGCCAAGACC
ATCATCGTGCAAGTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACAG
CGTAAGAGCATCCACATCGGCCCCGCGCGCCTTCTACGCCACCGGCGACATCATCGGC
GACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG
ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAACAGCAGC
ATCGGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC
TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGAACAAGACC
AAGGAGAACGACACCATCATCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG
GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAAT
ATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC
GAGACCTTCCGCCCCGCGCGGCAACATGAAGGACAAGTGGCGCAGCGAGCTGTACAAG
TACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGCCCCCACCAGGCCAAGCGCCGCGTG
GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGTTCTCGGCTTCTTGGGCGCC
GCCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACGGCCCCGCGAGCTGCTG
AGCGGCATCGTGACGAGCAGAGAACAACCTGCTGCGCGCCATCGAGGCCCGAGCAGCACTG
CTGCAGCTGACCGTGTGGGCGATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGC
TACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACC
ACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAAC
ATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTACAACCTG
ATCGAGATCGCCCAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAG
TGGGCCAGCCTGTGGAAGTGGTTCGACATCAACCAACTGGCTGTGGTACATCCGCATCTTC
ATCATGATCGTGCGCGCCCTGATCGGCCTGCGCATCGTGTTGCGCGTGCTGAGCATCGTG
AACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGCTGCCCGCCAGCGC
GGCCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGGACCGCGACCGCAGC
AACCGCCTGGTGACGGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCCTG
TTCAGCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGCCCGCATCGTGAGCTGCTG
GGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGTGGAACCTGCTGCAGTACTGGAGCCAG
GAGCTGAAGAGCAGCGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCCGAGGGC
ACCGACCGCATCATCGAGATCGTGACGCGCATCTTCCGCGCCGTGATCCACATCCCCCGC
CGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGAGAATTC

FIG. 61A

(SEQ ID NO:73)

SUBSTITUTE SHEET (RULE 26)

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CGCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCCTAACGTTACTGGCCGAAGCCGC
TTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTT
GGCAATGTGAGGGCCCCGAAACCTGGCCCTGTCTTCTTGACGAGCATTCTAGGGGTCTT
TCCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCTCTG
GAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCCTTGCAGGCAGCGGAACCCCCCA
CCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATAACCTGCAAAGGCG
GCACAACCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCC
TCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTTGTATGGGATCT
GATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTA
GGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATAATACCATGGGCGC
CCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAAGTGGGAGAAGATCCGCCTGCGCCC
CGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGGGCCAGCCGCGAGCTGGAGCG
CTTCGCGCTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCCGCCAGATCCTGGGCCA
GCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGCAGCCTGTACAACACCGTGGC
CACCTGTACTGCGTGACACGCGCATCGACGTCAAGGACACCAAGGAGGCCCTGGAGAA
GATCGAGGAGGAGCAGAACAAGTCCAAGAAGAAGGCCAGCAGGCCGCCGCCGCCGCCG
CACCGGCAACAGCAGCCAGGTGAGCCAGAACTACCCCATCGTGCAGAACCTGCAGGGCCA
GATGGTGCACACGAGCCATCAGCCCCCGCACCTGAACGCCTGGGTGAAGGTGGTGGAGGA
GAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTACGCGCCTGAGCGAGGGCGCCACCCC
CCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCAGATGCT
GAAGGAGACCATCAACGAGGAGGCCGCCGAGTGGGACCGCGTGCACCCCGTGCACGCCGG
CCCCATCGCCCCCGGCCAGATGCGCGAGCCCCCGCGGCAGCGACATCGCCCGGCACCACCAG
CACCTGTCAGGAGCAGATCGGCTGGATGACCAACAACCCCCCATCCCCGTGGGCGAGAT
CTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCGTGCGGATGTACAGCCCCACCAG
CATCCTGGACATCCGCCAGGGCCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGCTTCTA
CAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGAAGAACTGGATGACCGAGACCTT
GCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTGAAGGCTCTCGGCCCGCGGC
CACCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGGCCCGGCCACAAGGCCCG
CGTGTGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCGACCATCATGATGCAGCGCGG
CAACTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAACTGCGGCAAGGAGGGCCACAC
CGCCAGGAAGTCCGCGCCCCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCCGCGAGGGCCA
CCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGCAAGATCTGGCCAGCTA
CAAGGGCCGCCCCGGAACCTTCTGTCAGAGCCGCCCCGAGCCACCGCCCCCCCCGAGGA
GAGCTTCCGCTTCGGCGAGGAGAAGACCCCCAGCCAGAAGCAGGAGCCCATCGACAA
GGAGCTGTACCCCTGACCAGCCTGCGCAGCCTGTTTCGGCAACGACCCAGCAGCCAGTA
AGAATTCACTCGAGCAAGTCTAGA

FIG. 61B

(SEQ ID NO:73)

SUBSTITUTE SHEET (RULE 26)

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gp160mod.SF162.gag.modSF2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTTCGCCAGCGCCGTGGAGAAGCTGTGGGTGACCGTGTACTACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCCTAC
GACACCGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCC
CCAGGAGATCGTGCTGGAGAACGTGACCGAGAATTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGACCTGCACTGCACCAACCTGAAGAACGCCACCAACAC
CAAGAGCAGCAACTGGAAGGAGATGGACCGCGCGAGATCAAGAACTGCAGCTTCAAGG
TGACCACCAGCATCCGCAACAGATGCAGAAGGAGTACGCCCTGTTCTACAAGCTGGAC
GTGGTGCCCATCGACAACGACAACACCAGCTACAAGCTGATCAACTGCAACACCAGCGT
GATCACCCAGGCCTGCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCC
CCGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCGGCCCTGCG
ACCAACGTGAGCACCCTGTCAGTGACCCACGGCATCCGCCCCGTGGTGAGCACCAGCT
GCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGTGGTGATCCGCAGCGAGAATTACCGG
ACAACGCCAAGACCATCATCGTGACGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGC
CCCAACAACAACCCCGCAAGAGCATCACCATCGGCCCGGCCGCGCCTTCTACGCCAC
CGCGGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGGCGAGAAGTGGA
ACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCAGTTCCGCAACAAGACCATC
GTGTTCAAGCAGAGCAGCGCGCGGACCCCGAGATCGTGATGCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACAGCACCCAGCTGTTCAACAGCACCTGGAACAACACCA
TCGGCCCCAACAACACCAACGGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATC
AACCGCTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCG
CTGCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAGATCAGCA
ACACCACCGAGATCTTCCGCCCCGGCGGCGGCGACATGCGCGACAACCTGGCGCAGCGAG
CTGTACAAGTACAAGGTGGTGAAGATCGAGCCCTGGGCGTGGCCCCCACCAGGCCAA
GCGCCGCGTGGTGACGCGGAGAAGCGCGCCGTGACCTGGGCGCCATGTTCTGGGCT
TCCTGGGCGCCGCCGCGCAGCACCATGGGCGCCCCGAGCCTGACCCTGACCGTGACGGC
CGCCAGCTGCTGAGCGGCATCGTGACGAGCAGACAACCTGCTGCGCGCCATCGAGGC
CCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCCGCGTG
TGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGC
AAGCTGATCTGCACCACCGCCGTGCCCTGGAACGCCAGCTGGAGCAACAAGAGCCTGGA
CCAGATCTGGAACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGACAACCTACACCA
ACCTGATCTACACCCTGATCGAGGAGAGCCAGAACCAGCAGGAGAAGAACGAGCAGGAG
CTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTTCGACATCAGCAAGTGGCT
GTGGTACATCAAGATCTTCATCATGATCGTGGGCGGCCTGGTGGGCCTGCGCATCGTG
TCACCGTGCTGAGCATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCCTGAGCTTCCAG
ACCCGCTTCCCCGCCCCCGCGGCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGG
CGAGCGGACCGCGACCGCAGCAGCCCCCTGGTGACGGCCTGCTGGCCCTGATCTGGG
ACGACCTGCGCAGCCTGTGCCTGTTACGCTACCACCGCCTGCGCGACCTGATCCTGATC
GCCGCCCGCATCGTGAGCTGCTGGGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGGG
CAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGCGCCGTGAGCCTGTTTCGACG
CCATCGCCATCGCCGTGGCCGAGGACCGACCGCATCATCGAGGTGGCCCAGCGCATC
GGCCGCGCCTTCTGACATCCCCCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCT

FIG. 62A

(SEQ ID NO:74)

SUBSTITUTE SHEET (RULE 26)

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gp160modUS4.delV1/V2.gag.modSF2

GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGA
GCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGGTG
CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAG
GCCGAGGCCACACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG
GAGGTGAACCTGACCAACGTGACCGAGAACCTCAACATGTGGAAGAACAACATGGTGGAG
CAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGGGCGCC
GGCCAGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCC
GGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCCCTGCAAGAAC
GTGAGCACCGTGCACTGCACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTG
AACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACCGACAACGCC
AAGACCATCATCGTGCACTGAAAGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAAAC
AACACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATC
ATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTC
GAGCAGATCGTGGAAGAAGCTGCGCGAGCAGTTCCGGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGGCGAGTTC
TTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGAAC
AAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATG
TGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGC
AGCAATATTACCGGCTGCTGCTGACCGCGCAGCGCGGCACCAACAACAACCGCACCAAC
GACACCGAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTG
TACAAGTACAAGGTGGTGCATCGAGCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGC
CGCGTGGTGCAGCGGAGAAGCGCGCCGTGGGCGCTGGGCGCCCTGTTTCATCGGCTTCCTG
GGCGCCGCCGGGAGCACCATGGGCGCCGCTCCGTGACCCCTGACCGTGACGGCCCCGCGAG
CTGCTGAGCGGCATCGTGACGAGCAGACAACCTGCTGCGCGCCATCGAGGCCCGAGCAG
CACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTG
GAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATC
TGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGG
GACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTAC
AACCTGATCGAGATCGCCAGAACAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTG
GACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGGCTGTGGTACATCCGC
ATCTTCATCATGATCGTGGGCGGCCCTGATCGGCCTGCGCATCGTCTTCGCCGTGCTGAGC
ATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGCCTGCCCGCC
CAGCGCGGCCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGAC
CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTG
TGCTTGTTCAGCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG
CTGCTGGGCGCGCGGCTGGGAGGCCCTGAAGTACTGGTGGAACTGCTGCAGTACTGG
AGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC
GAGGGCACCGACCGCATCATCGAGATCGTGACGCGCATCTTCCGCGCCGTGATCCACATC
CCCCGCCGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA
GAATTCCGCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCCTAACGTTACTGGCCGA
AGCCGCTTGGAAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCG
TCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGG
GGTCTTTCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTT

FIG. 63A

(SEQ ID NO:75)

SUBSTITUTE SHEET (RULE 26)

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CCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAAC
CCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCA
AAGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGG
CTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTTGTATG
GGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAA
CGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATAATACCAT
GGGCGCCCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGTGGGAGAAGATCCGCCT
GCGCCCCGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGGGCCAGCCGCGAGCT
GGAGCGCTTCGCGGTGAACCCCGGCCTGCTGGAGACCAGCGAGGGCTGCCGCCAGATCCT
GGGCCAGCTGCAGCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGCAGCCTGTACAACAC
CGTGGCCACCCTGTACTGCGTGCACCAGCGCATCGACGTCAAGGACACCAAGGAGGCCCT
GGAGAAGATCGAGGAGGAGCAGAACAAGTCCAAGAAGAAGGCCAGCAGGCCGCCGCCGC
CGCCGGCACC GGCAACAGCAGCCAGGTGAGCCAGAACTACCCCATCGTGCAGAACCTGCA
GGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCCCTGAACGCCTGGGTGAAGGTGGT
GGAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTACGCGCCCTGAGCGAGGGCGC
CACCCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCA
GATGCTGAAGGAGACCATCAACGAGGAGGCCGCCGAGTGGGACCGCGTGCACCCCGTGCA
CGCCGGCCCCATCGCCCCCGGCCAGATGCGCGAGCCCCGCGGCAGCGACATCGCCGGCAC
CACCAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCAACAACCCCCCATCCCCGTGGG
CGAGATCTACAAGCGGTGGATCATCTGGGCCTGAACAAGATCGTGCGGATGTACAGCCC
CACCAGCATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCG
CTTCTACAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGAAGAACTGGATGACCGA
GACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTGAAGGCTCTCGGCCC
CGCGGCCACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGGCCCGGCCACAA
GGCCCGCGTGTGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCGACCATCATGATGCA
GCGCGGCAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAACTGCGGCAAGGAGGG
CCACACCGCCAGGAAGTCCCGCGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCCGCGA
GGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGCAAGATCTGGCC
CAGCTACAAGGGCCGCCCGGCAACTTCTGCAGAGCCGCCCGAGCCACCGCCCCCCC
CGAGGAGAGCTTCCGCTTCGCGCGAGGAGAAGACCACCCCGAGCCAGAAGCAGGAGCCCAT
CGACAAGGAGCTGTACCCCTGACCAGCCTGCGCAGCCTGTTGCGCAACGACCCCGAGCAG
CCAGTAAGAATTCAGACTCGAGCAAGTCTAGA

FIG. 63B

(SEQ ID NO:75)

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gp160.modSF162.delV2.gag.modSF2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGA
GCAGTCTTCGTTTCGCCCAGCGCCGTGGAGAAGCTGTGGGTGACCGTGTACTACGGCGTG
CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCCTACGAC
ACCGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG
GAGATCGTGCTGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG
CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG
ACCCCCCTGTGCGTGACCTGCACTGCACCAACCTGAAGAACGCCACCAACACCAAGAGC
AGCAACTGGAAGGAGATGGACCGCGGCGAGATCAAGAACTGCAGCTTCAAGGTGGGCGCC
GGCAAGCTGATCAACTGCAACACCAGCGTGATCACCAGGCCTGCCCCAAGGTGAGCTTC
GAGCCCATCCCCATCCACTACTGCGCCCCCGCGGCTTCGCCATCCTGAAGTGCAACGAC
AAGAAGTTCAACGGCAGCGGCCCTGCACCAACGTGAGCACCCTGCGTGCAGTGCACCCACGGC
ATCCGCCCCGTGGTGAGCACCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGTG
GTGATCCGCGAGCGAGAAGTTCAACGACAACGCCAAGACCATCATCGTGAGCTGAAGGAG
AGCGTGAGATCAACTGCACCCGCCCCAACAACAACACCCGCAAGAGCATCACCATCGGC
CCCCGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTGC
AACATCAGCGGCGAGAAGTGGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCC
CAGTTCGGCAACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGTG
ATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCCAGCTGTTCAAC
AGCACCTGGAACAACACCATCGGCCCCAACAACACCAACGGCACCATCACCTGCCCTGC
CGCATCAAGCAGATCATCAACCGCTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCC
ATCCGCGGCCAGATCCGCTGCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGC
GGCAAGGAGATCAGCAACACCACCGAGATCTTCCGCCCCGCGGCGGCGACATGCGCGAC
AACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCCCTGGGCGTGGCC
CCCACCAAGGCCAAGCGCCGCGTGCTGTCAGCGCGAGAAGCGCGCCGTGACCTGGGCGCC
ATGTTCTTGGGCTTCTTGGGCGCGCGCGGCGAGCACCATGGGCGCCCCGAGCCTGACCCTG
ACCGTGCAGGCCCCGCCAGCTGCTGAGCGGCATCGTGTCAGCAGCAGAACAACTGCTGCGC
GCCATCGAGGCCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAG
GCCCCGCTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGCG
TGCAGCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGGAACGCCAGCTGGAGCAACAAG
AGCCTGGACCAGATCTGGAACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGACAAC
TACACCAACCTGATCTACACCCTGATCGAGGAGAGCCAGAACCAGCAGGAGAAGAACGAG
CAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAAGTGGTTTCGACATCAGCAAG
TGGCTGTGGTACATCAAGATCTTCATCATGATCGTGGGCGGCCTGGTGGGCCTGCGCATC
GTGTTACCGTGCTGAGCATCGTGAACCGCGTGCAGGAGGCTACAGCCCCCTGAGCTTC
CAGACCCGCTTCCCCGCCCCCGCGCGGCCGAGGCCATCGAGGAGGAGGGC
GGCGAGCGCGACCGCGACCGCAGCGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGG
GACGACCTGCGCGAGCCTGTGCCTGTTACGCTACCACCGCCTGCGCGACCTGATCCTGATC
GCCGCCCCGATCGTGAGCTGCTGGGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGGGC
AACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGCGCCGTGAGCCTGTTTCGACGCC
ATCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGGTGGCCCAGCGCATCGGC
CGCGCCTTCTTGACATCCCCCGCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCTGTAA
CTCGAGCAAGTCTAGAGAATTCCGCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCC
TAACGTTACTGGCCGAAGCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATT
TTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGAAACCTGGCCCTGTCTTCTT

FIG. 64A

(SEQ ID NO:76)

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GACGAGCATTTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGT
CGTGAAGGAAGCAGTTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCCT
TTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGT
ATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGT
GGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAA
GGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTA
GTCGAGGTTAAAAAACGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAA
AACACGATAATACCATGGGCGCCCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGT
GGGAGAAGATCCGCCTGCGCCCCGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGT
GGGCCAGCCGCGAGCTGGAGCGCTTCGCCGTGAACCCCGGCCTGCTGGAGACCAGCGAGG
GCTGCCCGCCAGATCCTGGGCCAGCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGC
GCAGCCTGTACAACACCGTGGCCACCCTGTACTGCGTGCACCAGCGCATCGACGTCAAGG
ACACCAAGGAGGCCCTGGAGAAGATCGAGGAGGAGCAGAACAAGTCCAAGAAGAAGGCC
AGCAGGCCCGCCGCCCGCCCGGCCACCGGCAACAGCAGCCAGGTGAGCCAGAATAACCCCA
TCGTGCAGAACCTGCAGGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCCCTGAACG
CCTGGGTGAAGGTGGTGGAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCG
CCCTGAGCGAGGGCGCCACCCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCC
ACCAGGCCCGCCATGCAGATGCTGAAGGAGACCATCAACGAGGAGGCCCGCCGAGTGGGACC
GCGTGCACCCCGTGCACGCCGGCCCCATCGCCCCCGGCCAGATGCGCGAGCCCCGCGGCA
GCGACATCGCCGGCACCACCAGCACCTGCAGGAGCAGATCGGCTGGATGACCAACAACC
CCCCCATCCCCGTGGGCGAGATCTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCG
TGCGGATGTACAGCCCCACCAGCATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCC
AGAACTGGATGACCGAGACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCC
TGAAGGCTCTCGGCCCCCGCGCCACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGG
GCGGCCCCCGGCCACAAGGCCCCGCTGCTGGCCGAGGCGATGAGCCAGGTGACGAACCCGG
CGACCATCATGATGCAGCGCGGCAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCA
ACTGCGGCAAGGAGGGCCACCGCCAGGAACTGCCGCGCCCCCGCAAGAAGGGCTGCT
GGCGCTGCGGCCGCGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCC
TGGGCAAGATCTGGCCCAGCTACAAGGGCCGCCCGGCAACTTCTGCAGAGCCGCCCCG
AGCCCACCGCCCCCCCCGAGGAGGCTTCCGCTTCGGCGAGGAGAAGACCACCCCCAGCC
AGAAGCAGGAGCCCATCGACAAGGAGCTGTACCCCTGACCAGCCTGCGCAGCCTGTTTCG
GCAACGACCCCGAGCAGCCAGTAAGAATTGAGACTCGAGCAAGTCTAGA

FIG. 64B

(SEQ ID NO:76)

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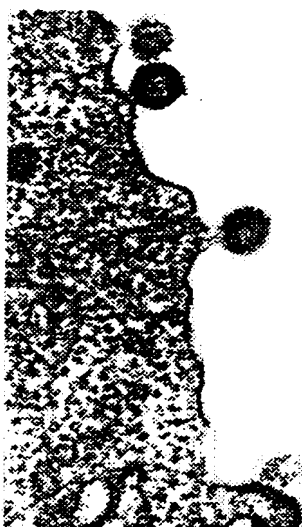


FIG. 65C



FIG. 65B

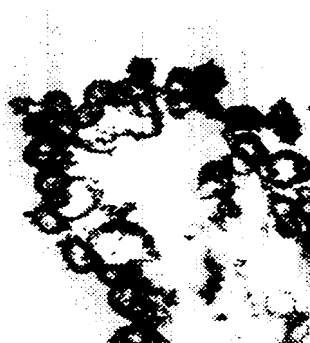


FIG. 65A



FIG. 65F



FIG. 65E



FIG. 65D

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| | | | |
|------------------------|-------|---|-----|
| gp160.modSF162 | 1 | GAATTCGCCACCACCATGGATGCAATGAAGAGAGAGGGCTCTGCTGTGTGCTGCT | 50 |
| gp160.modSF162.delV2 | (1) | GAATTCGCCACCACCATGGATGCAATGAAGAGAGAGGGCTCTGCTGTGTGCTGCT | |
| gp160.modSF162.delV1V2 | (1) | GAATTCGCCACCACCATGGATGCAATGAAGAGAGAGGGCTCTGCTGTGTGCTGCT | |
| gp140.modSF162 | (1) | GAATTCGCCACCACCATGGATGCAATGAAGAGAGAGGGCTCTGCTGTGTGCTGCT | |
| gp140.mut.modSF162 | (1) | GAATTCGCCACCACCATGGATGCAATGAAGAGAGAGGGCTCTGCTGTGTGCTGCT | |
| gp140.mut7.modSF162 | (1) | GAATTCGCCACCACCATGGATGCAATGAAGAGAGAGGGCTCTGCTGTGTGCTGCT | |
| gp140.mut8.modSF162 | (1) | GAATTCGCCACCACCATGGATGCAATGAAGAGAGAGGGCTCTGCTGTGTGCTGCT | |
| gp120.modSF162 | (1) | GAATTCGCCACCACCATGGATGCAATGAAGAGAGAGGGCTCTGCTGTGTGCTGCT | 100 |
| Consensus | 51 | GCTGTGTGGAGCAGCTCTCGTTTCGCCACGCCCGTGGAGAAGCTGTGGG | |
| gp160.modSF162 | (51) | GCTGTGTGGAGCAGCTCTCGTTTCGCCACGCCCGTGGAGAAGCTGTGGG | |
| gp160.modSF162.delV2 | (51) | GCTGTGTGGAGCAGCTCTCGTTTCGCCACGCCCGTGGAGAAGCTGTGGG | |
| gp160.modSF162.delV1V2 | (51) | GCTGTGTGGAGCAGCTCTCGTTTCGCCACGCCCGTGGAGAAGCTGTGGG | |
| gp140.modSF162 | (51) | GCTGTGTGGAGCAGCTCTCGTTTCGCCACGCCCGTGGAGAAGCTGTGGG | |
| gp140.mut.modSF162 | (51) | GCTGTGTGGAGCAGCTCTCGTTTCGCCACGCCCGTGGAGAAGCTGTGGG | |
| gp140.mut7.modSF162 | (51) | GCTGTGTGGAGCAGCTCTCGTTTCGCCACGCCCGTGGAGAAGCTGTGGG | |
| gp140.mut8.modSF162 | (51) | GCTGTGTGGAGCAGCTCTCGTTTCGCCACGCCCGTGGAGAAGCTGTGGG | |
| gp120.modSF162 | (51) | GCTGTGTGGAGCAGCTCTCGTTTCGCCACGCCCGTGGAGAAGCTGTGGG | 150 |
| Consensus | 101 | TGACCGTGTAACGGCGTGCCCGTGTGGAAGGAGGCCACCCACCCCTG | |
| gp160.modSF162 | (101) | TGACCGTGTAACGGCGTGCCCGTGTGGAAGGAGGCCACCCACCCCTG | |
| gp160.modSF162.delV2 | (101) | TGACCGTGTAACGGCGTGCCCGTGTGGAAGGAGGCCACCCACCCCTG | |
| gp160.modSF162.delV1V2 | (101) | TGACCGTGTAACGGCGTGCCCGTGTGGAAGGAGGCCACCCACCCCTG | |
| gp140.modSF162 | (101) | TGACCGTGTAACGGCGTGCCCGTGTGGAAGGAGGCCACCCACCCCTG | |
| gp140.mut.modSF162 | (101) | TGACCGTGTAACGGCGTGCCCGTGTGGAAGGAGGCCACCCACCCCTG | |
| gp140.mut7.modSF162 | (101) | TGACCGTGTAACGGCGTGCCCGTGTGGAAGGAGGCCACCCACCCCTG | |
| gp140.mut8.modSF162 | (101) | TGACCGTGTAACGGCGTGCCCGTGTGGAAGGAGGCCACCCACCCCTG | |
| gp120.modSF162 | (101) | TGACCGTGTAACGGCGTGCCCGTGTGGAAGGAGGCCACCCACCCCTG | |
| Consensus | | | |

FIG. 66A-1

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| | | | | |
|------------------------|-------|---|-----|-----|
| | | | 151 | 200 |
| gp160.modSF162 | (151) | TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG | | |
| gp160.modSF162.delV2 | (151) | TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG | | |
| gp160.modSF162.delV1V2 | (151) | TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG | | |
| gp140.modSF162 | (151) | TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG | | |
| gp140.mut.modSF162 | (151) | TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG | | |
| gp140.mut7.modSF162 | (151) | TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG | | |
| gp140.mut8.modSF162 | (151) | TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG | | |
| gp120.modSF162 | (151) | TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG | | |
| Consensus | (151) | TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG | | |
| | | | 201 | 250 |
| gp160.modSF162 | (201) | GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGATCGTGC | | |
| gp160.modSF162.delV2 | (201) | GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGATCGTGC | | |
| gp160.modSF162.delV1V2 | (201) | GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGATCGTGC | | |
| gp140.modSF162 | (201) | GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGATCGTGC | | |
| gp140.mut.modSF162 | (201) | GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGATCGTGC | | |
| gp140.mut7.modSF162 | (201) | GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGATCGTGC | | |
| gp140.mut8.modSF162 | (201) | GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGATCGTGC | | |
| gp120.modSF162 | (201) | GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGATCGTGC | | |
| Consensus | (201) | GGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGATCGTGC | | |
| | | | 251 | 300 |
| gp160.modSF162 | (251) | TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG | | |
| gp160.modSF162.delV2 | (251) | TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG | | |
| gp160.modSF162.delV1V2 | (251) | TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG | | |
| gp140.modSF162 | (251) | TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG | | |
| gp140.mut.modSF162 | (251) | TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG | | |
| gp140.mut7.modSF162 | (251) | TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG | | |
| gp140.mut8.modSF162 | (251) | TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG | | |

FIG. 66A-2

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gp120.modSF162
 Consensus
 (251) TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAAACATGGTGGAG
 (251) TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAAACATGGTGGAG 350
 301
 (301) CAGATGCACGAGGACATCATCAGCCCTGTGGACCAAGAGCCTGAAGCCCTG
 (301) CAGATGCACGAGGACATCATCAGCCCTGTGGACCAAGAGCCTGAAGCCCTG
 (301) CAGATGCACGAGGACATCATCAGCCCTGTGGACCAAGAGCCTGAAGCCCTG
 (301) CAGATGCACGAGGACATCATCAGCCCTGTGGACCAAGAGCCTGAAGCCCTG
 (301) CAGATGCACGAGGACATCATCAGCCCTGTGGACCAAGAGCCTGAAGCCCTG
 (301) CAGATGCACGAGGACATCATCAGCCCTGTGGACCAAGAGCCTGAAGCCCTG
 (301) CAGATGCACGAGGACATCATCAGCCCTGTGGACCAAGAGCCTGAAGCCCTG
 (301) CAGATGCACGAGGACATCATCAGCCCTGTGGACCAAGAGCCTGAAGCCCTG
 (301) CAGATGCACGAGGACATCATCAGCCCTGTGGACCAAGAGCCTGAAGCCCTG
 (301) CAGATGCACGAGGACATCATCAGCCCTGTGGACCAAGAGCCTGAAGCCCTG 400
 351
 (351) CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA
 (351) CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA
 (351) CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA
 (351) CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA
 (351) CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA
 (351) CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA
 (351) CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA
 (351) CGTGAAGCTGACCCCCCTGTGCGTGACCCCTGCACCTGCACCAACCTGAAGA 450
 401
 (401) ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGGGCGAG
 (401) ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGGGCGAG
 (375) -----
 (401) ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGGGCGAG
 (401) ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGGGCGAG
 (401) ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGGGCGAG
 (401) ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGGGCGAG
 (401) ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGGGCGAG
 (401) ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGGGCGAG
 (401) ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGGGCGAG
 (401) ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGGGCGAG
 Consensus
 gp160.modSF162
 gp160.modSF162.delV2
 gp160.modSF162.delV1V2
 gp140.modSF162
 gp140.mut.modSF162
 gp140.mut7.modSF162
 gp140.mut8.modSF162
 gp120.modSF162
 Consensus
 gp160.modSF162
 gp160.modSF162.delV2
 gp160.modSF162.delV1V2
 gp140.modSF162
 gp140.mut.modSF162
 gp140.mut7.modSF162
 gp140.mut8.modSF162
 gp120.modSF162
 Consensus
 gp160.modSF162
 gp160.modSF162.delV2
 gp160.modSF162.delV1V2
 gp140.modSF162
 gp140.mut.modSF162
 gp140.mut7.modSF162
 gp140.mut8.modSF162
 gp120.modSF162
 Consensus

FIG. 66A-3

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| | | | |
|------------------------|-------|--|-----|
| gp160.modSF162 | (451) | ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA | 500 |
| gp160.modSF162.delV2 | (451) | ATCAAGAACTGCAGCTTCAAGGTGGC----- | |
| gp160.modSF162.delV1V2 | (376) | -----GGC----- | |
| gp140.modSF162 | (451) | ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA | |
| gp140.mut.modSF162 | (451) | ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA | |
| gp140.mut7.modSF162 | (451) | ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA | |
| gp140.mut8.modSF162 | (451) | ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA | |
| gp120.modSF162 | (451) | ATCAAGAACTGCAGCTTCAAGGTGACCAACAGCATCCGCAACAAGATGCA | |
| Consensus | 501 | | |
| gp160.modSF162 | (501) | GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG | |
| gp160.modSF162.delV2 | (478) | -----GCC-----GG----- | |
| gp160.modSF162.delV1V2 | (379) | -----GCC-----GG----- | |
| gp140.modSF162 | (501) | GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG | |
| gp140.mut.modSF162 | (501) | GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG | |
| gp140.mut7.modSF162 | (501) | GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG | |
| gp140.mut8.modSF162 | (501) | GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG | |
| gp120.modSF162 | (501) | GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG | |
| Consensus | 551 | | 600 |
| gp160.modSF162 | (551) | ACACACACAGCTACAAGCTGATCAACTGCAACACACAGCGTGATCACCAG | |
| gp160.modSF162.delV2 | (492) | -----CAAGCTGATCAACTGCAACACACAGCGTGATCACCAG | |
| gp160.modSF162.delV1V2 | (384) | -----CAACTGCCAGACCCAGCGTGATCACCAG | |
| gp140.modSF162 | (551) | ACAACACAGCTACAAGCTGATCAACTGCAACACACAGCGTGATCACCAG | |
| gp140.mut.modSF162 | (551) | ACAACACAGCTACAAGCTGATCAACTGCAACACACAGCGTGATCACCAG | |

FIG. 66A-4

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| | | | |
|------------------------|-------|---|-----|
| gp140.mut7.modSF162 | (551) | ACAAACACAGCTACAAGCTGATCAACTGCAACACACAGCGTGATCACCCAG | 650 |
| gp140.mut8.modSF162 | (551) | ACAAACACAGCTACAAGCTGATCAACTGCAACACACAGCGTGATCACCCAG | |
| gp120.modSF162 | (551) | ACAAACACAGCTACAAGCTGATCAACTGCAACACACAGCGTGATCACCCAG | |
| Consensus | (551) | ACAAACACAGCTACAAGCTGATCAACTGCAACACACAGCGTGATCACCCAG | |
| gp160.modSF162 | (601) | GCCTGCCCCAAGGTGAGCTTCGAGGCCATCCCCATCCCACTACTGCGCCCC | 601 |
| gp160.modSF162.delV2 | (520) | GCCTGCCCCAAGGTGAGCTTCGAGGCCATCCCCATCCCACTACTGCGCCCC | |
| gp160.modSF162.delV1V2 | (412) | GCCTGCCCCAAGGTGAGCTTCGAGGCCATCCCCATCCCACTACTGCGCCCC | |
| gp140.modSF162 | (601) | GCCTGCCCCAAGGTGAGCTTCGAGGCCATCCCCATCCCACTACTGCGCCCC | |
| gp140.mut.modSF162 | (601) | GCCTGCCCCAAGGTGAGCTTCGAGGCCATCCCCATCCCACTACTGCGCCCC | |
| gp140.mut7.modSF162 | (601) | GCCTGCCCCAAGGTGAGCTTCGAGGCCATCCCCATCCCACTACTGCGCCCC | |
| gp140.mut8.modSF162 | (601) | GCCTGCCCCAAGGTGAGCTTCGAGGCCATCCCCATCCCACTACTGCGCCCC | |
| gp120.modSF162 | (601) | GCCTGCCCCAAGGTGAGCTTCGAGGCCATCCCCATCCCACTACTGCGCCCC | |
| Consensus | (601) | GCCTGCCCCAAGGTGAGCTTCGAGGCCATCCCCATCCCACTACTGCGCCCC | 700 |
| gp160.modSF162 | (651) | CGCCGGCTTCGCCATCCTGAAGTGCAACGACAGAAGTTCACACGGCAGCG | 651 |
| gp160.modSF162.delV2 | (570) | CGCCGGCTTCGCCATCCTGAAGTGCAACGACAGAAGTTCACACGGCAGCG | |
| gp160.modSF162.delV1V2 | (462) | CGCCGGCTTCGCCATCCTGAAGTGCAACGACAGAAGTTCACACGGCAGCG | |
| gp140.modSF162 | (651) | CGCCGGCTTCGCCATCCTGAAGTGCAACGACAGAAGTTCACACGGCAGCG | |
| gp140.mut.modSF162 | (651) | CGCCGGCTTCGCCATCCTGAAGTGCAACGACAGAAGTTCACACGGCAGCG | |
| gp140.mut7.modSF162 | (651) | CGCCGGCTTCGCCATCCTGAAGTGCAACGACAGAAGTTCACACGGCAGCG | |
| gp140.mut8.modSF162 | (651) | CGCCGGCTTCGCCATCCTGAAGTGCAACGACAGAAGTTCACACGGCAGCG | |
| gp120.modSF162 | (651) | CGCCGGCTTCGCCATCCTGAAGTGCAACGACAGAAGTTCACACGGCAGCG | |
| Consensus | (651) | CGCCGGCTTCGCCATCCTGAAGTGCAACGACAGAAGTTCACACGGCAGCG | 750 |
| gp160.modSF162 | (701) | GCCCTGCACCAACGTGAGCACCGTGAGTGCAACCCACGGCATCCGCCCC | 701 |
| gp160.modSF162.delV2 | (620) | GCCCTGCACCAACGTGAGCACCGTGAGTGCAACCCACGGCATCCGCCCC | |
| gp160.modSF162.delV1V2 | (512) | GCCCTGCACCAACGTGAGCACCGTGAGTGCAACCCACGGCATCCGCCCC | |
| gp140.modSF162 | (701) | GCCCTGCACCAACGTGAGCACCGTGAGTGCAACCCACGGCATCCGCCCC | |
| gp140.mut.modSF162 | (701) | GCCCTGCACCAACGTGAGCACCGTGAGTGCAACCCACGGCATCCGCCCC | |
| gp140.mut7.modSF162 | (701) | GCCCTGCACCAACGTGAGCACCGTGAGTGCAACCCACGGCATCCGCCCC | |
| gp140.mut8.modSF162 | (701) | GCCCTGCACCAACGTGAGCACCGTGAGTGCAACCCACGGCATCCGCCCC | |
| gp120.modSF162 | (701) | GCCCTGCACCAACGTGAGCACCGTGAGTGCAACCCACGGCATCCGCCCC | |
| Consensus | (701) | GCCCTGCACCAACGTGAGCACCGTGAGTGCAACCCACGGCATCCGCCCC | |

FIG. 66A-5

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| | 751 | 800 |
|------------------------|-------|---|
| gp160.modSF162 | (751) | GTGTTAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCCGAGGAGGGCGT |
| gp160.modSF162.delV2 | (670) | GTGTTAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCCGAGGAGGGCGT |
| gp160.modSF162.delV1V2 | (562) | GTGTTAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCCGAGGAGGGCGT |
| gp140.modSF162 | (751) | GTGTTAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCCGAGGAGGGCGT |
| gp140.mut.modSF162 | (751) | GTGTTAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCCGAGGAGGGCGT |
| gp140.mut7.modSF162 | (751) | GTGTTAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCCGAGGAGGGCGT |
| gp140.mut8.modSF162 | (751) | GTGTTAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCCGAGGAGGGCGT |
| gp120.modSF162 | (751) | GTGTTAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCCGAGGAGGGCGT |
| Consensus | | 850 |
| gp160.modSF162 | (801) | GGTGATCCGCGAGCGAGAACTTCACCGACAAACGCCAAGACCATCATCTGTCG |
| gp160.modSF162.delV2 | (720) | GGTGATCCGCGAGCGAGAACTTCACCGACAAACGCCAAGACCATCATCTGTCG |
| gp160.modSF162.delV1V2 | (612) | GGTGATCCGCGAGCGAGAACTTCACCGACAAACGCCAAGACCATCATCTGTCG |
| gp140.modSF162 | (801) | GGTGATCCGCGAGCGAGAACTTCACCGACAAACGCCAAGACCATCATCTGTCG |
| gp140.mut.modSF162 | (801) | GGTGATCCGCGAGCGAGAACTTCACCGACAAACGCCAAGACCATCATCTGTCG |
| gp140.mut7.modSF162 | (801) | GGTGATCCGCGAGCGAGAACTTCACCGACAAACGCCAAGACCATCATCTGTCG |
| gp140.mut8.modSF162 | (801) | GGTGATCCGCGAGCGAGAACTTCACCGACAAACGCCAAGACCATCATCTGTCG |
| gp120.modSF162 | (801) | GGTGATCCGCGAGCGAGAACTTCACCGACAAACGCCAAGACCATCATCTGTCG |
| Consensus | | 900 |
| gp160.modSF162 | (851) | AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCCAACAACAACACC |
| gp160.modSF162.delV2 | (770) | AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCCAACAACAACACC |
| gp160.modSF162.delV1V2 | (662) | AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCCAACAACAACACC |

FIG. 66A-6

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| | | | |
|------------------------|-------|--|------|
| gp140.modSF162 | (851) | AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCGCCCAACAACAACACC | 950 |
| gp140.mut.modSF162 | (851) | AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCGCCCAACAACAACACC | |
| gp140.mut7.modSF162 | (851) | AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCGCCCAACAACAACACC | |
| gp140.mut8.modSF162 | (851) | AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCGCCCAACAACAACACC | |
| gp120.modSF162 | (851) | AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCGCCCAACAACAACACC | |
| Consensus | | | 950 |
| gp160.modSF162 | (901) | CGCAAGAGCATCAACATCGGCCCGCGCGCGCTTCTACGCCACCGGCGGA | 901 |
| gp160.modSF162.delV2 | (820) | CGCAAGAGCATCAACATCGGCCCGCGCGCGCTTCTACGCCACCGGCGGA | |
| gp160.modSF162.delV1V2 | (712) | CGCAAGAGCATCAACATCGGCCCGCGCGCGCTTCTACGCCACCGGCGGA | |
| gp140.modSF162 | (901) | CGCAAGAGCATCAACATCGGCCCGCGCGCGCTTCTACGCCACCGGCGGA | |
| gp140.mut.modSF162 | (901) | CGCAAGAGCATCAACATCGGCCCGCGCGCGCTTCTACGCCACCGGCGGA | |
| gp140.mut7.modSF162 | (901) | CGCAAGAGCATCAACATCGGCCCGCGCGCGCTTCTACGCCACCGGCGGA | |
| gp140.mut8.modSF162 | (901) | CGCAAGAGCATCAACATCGGCCCGCGCGCGCTTCTACGCCACCGGCGGA | |
| gp120.modSF162 | (901) | CGCAAGAGCATCAACATCGGCCCGCGCGCGCTTCTACGCCACCGGCGGA | |
| Consensus | | | 1000 |
| gp160.modSF162 | (951) | CATCATCGGCGACATCCGCCAGGCGCCACTGCAACATCAGCGGCGGAGAAGT | 951 |
| gp160.modSF162.delV2 | (870) | CATCATCGGCGACATCCGCCAGGCGCCACTGCAACATCAGCGGCGGAGAAGT | |
| gp160.modSF162.delV1V2 | (762) | CATCATCGGCGACATCCGCCAGGCGCCACTGCAACATCAGCGGCGGAGAAGT | |
| gp140.modSF162 | (951) | CATCATCGGCGACATCCGCCAGGCGCCACTGCAACATCAGCGGCGGAGAAGT | |
| gp140.mut.modSF162 | (951) | CATCATCGGCGACATCCGCCAGGCGCCACTGCAACATCAGCGGCGGAGAAGT | |
| gp140.mut7.modSF162 | (951) | CATCATCGGCGACATCCGCCAGGCGCCACTGCAACATCAGCGGCGGAGAAGT | |
| gp140.mut8.modSF162 | (951) | CATCATCGGCGACATCCGCCAGGCGCCACTGCAACATCAGCGGCGGAGAAGT | |
| gp120.modSF162 | (951) | CATCATCGGCGACATCCGCCAGGCGCCACTGCAACATCAGCGGCGGAGAAGT | |
| Consensus | | | 951 |

FIG. 66A-7

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| | | | |
|------------------------|--------|------|------|
| gp160.modSF162 | (1001) | 1001 | 1050 |
| gp160.modSF162.delV2 | (920) | | |
| gp160.modSF162.delV1V2 | (812) | | |
| gp140.modSF162 | (1001) | | |
| gp140.modSF162 | (1001) | | |
| gp140.mut.modSF162 | (1001) | | |
| gp140.mut7.modSF162 | (1001) | | |
| gp140.mut8.modSF162 | (1001) | | |
| gp120.modSF162 | (1001) | | |
| Consensus | (1001) | | |
| gp160.modSF162 | (1051) | 1051 | |
| gp160.modSF162.delV2 | (970) | | |
| gp160.modSF162.delV1V2 | (862) | | |
| gp140.modSF162 | (1051) | | |
| gp140.mut.modSF162 | (1051) | | |
| gp140.mut7.modSF162 | (1051) | | |
| gp140.mut8.modSF162 | (1051) | | |
| gp120.modSF162 | (1051) | | |
| Consensus | (1051) | | |
| gp160.modSF162 | (1101) | 1101 | |
| gp160.modSF162.delV2 | (1020) | | |
| gp160.modSF162.delV1V2 | (912) | | |
| gp140.modSF162 | (1101) | | |
| gp140.mut.modSF162 | (1101) | | |
| gp140.mut7.modSF162 | (1101) | | |
| gp140.mut8.modSF162 | (1101) | | |
| gp120.modSF162 | (1101) | | |
| Consensus | (1101) | | |
| gp160.modSF162 | (1151) | 1151 | |

FIG. 66A-8

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| | | | |
|------------------------|--------|--|------|
| gp160.modSF162.delV2 | (1070) | AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCAACAACACCAAC | 1250 |
| gp160.modSF162.delV1V2 | (962) | AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCAACAACACCAAC | |
| gp140.modSF162 | (1151) | AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCAACAACACCAAC | |
| gp140.modSF162 | (1151) | AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCAACAACACCAAC | |
| gp140.modSF162 | (1151) | AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCAACAACACCAAC | |
| gp140.modSF162 | (1151) | AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCAACAACACCAAC | |
| gp140.modSF162 | (1151) | AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCAACAACACCAAC | |
| gp120.modSF162 | (1151) | AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCAACAACACCAAC | |
| Consensus | (1151) | AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCAACAACACCAAC | 1250 |
| gp160.modSF162 | (1201) | GGCACCATCACCCCTGCCCTGCCGATCAAGCAGATCATCAACCGCTGGCA | 1201 |
| gp160.modSF162.delV2 | (1120) | GGCACCATCACCCCTGCCCTGCCGATCAAGCAGATCATCAACCGCTGGCA | |
| gp160.modSF162.delV1V2 | (1012) | GGCACCATCACCCCTGCCCTGCCGATCAAGCAGATCATCAACCGCTGGCA | |
| gp140.modSF162 | (1201) | GGCACCATCACCCCTGCCCTGCCGATCAAGCAGATCATCAACCGCTGGCA | |
| gp140.modSF162 | (1201) | GGCACCATCACCCCTGCCCTGCCGATCAAGCAGATCATCAACCGCTGGCA | |
| gp140.modSF162 | (1201) | GGCACCATCACCCCTGCCCTGCCGATCAAGCAGATCATCAACCGCTGGCA | |
| gp140.modSF162 | (1201) | GGCACCATCACCCCTGCCCTGCCGATCAAGCAGATCATCAACCGCTGGCA | |
| gp140.modSF162 | (1201) | GGCACCATCACCCCTGCCCTGCCGATCAAGCAGATCATCAACCGCTGGCA | |
| gp120.modSF162 | (1201) | GGCACCATCACCCCTGCCCTGCCGATCAAGCAGATCATCAACCGCTGGCA | |
| Consensus | (1201) | GGCACCATCACCCCTGCCCTGCCGATCAAGCAGATCATCAACCGCTGGCA | 1300 |
| gp160.modSF162 | (1251) | GGAGGTGGCAAGGCCATGTACGCCCCCCCCCATCCGGGCCAGATCCGCT | 1251 |
| gp160.modSF162.delV2 | (1170) | GGAGGTGGCAAGGCCATGTACGCCCCCCCCCATCCGGGCCAGATCCGCT | |
| gp160.modSF162.delV1V2 | (1062) | GGAGGTGGCAAGGCCATGTACGCCCCCCCCCATCCGGGCCAGATCCGCT | |
| gp140.modSF162 | (1251) | GGAGGTGGCAAGGCCATGTACGCCCCCCCCCATCCGGGCCAGATCCGCT | |
| gp140.modSF162 | (1251) | GGAGGTGGCAAGGCCATGTACGCCCCCCCCCATCCGGGCCAGATCCGCT | |
| gp140.modSF162 | (1251) | GGAGGTGGCAAGGCCATGTACGCCCCCCCCCATCCGGGCCAGATCCGCT | |
| gp140.modSF162 | (1251) | GGAGGTGGCAAGGCCATGTACGCCCCCCCCCATCCGGGCCAGATCCGCT | |
| gp140.modSF162 | (1251) | GGAGGTGGCAAGGCCATGTACGCCCCCCCCCATCCGGGCCAGATCCGCT | |
| gp120.modSF162 | (1251) | GGAGGTGGCAAGGCCATGTACGCCCCCCCCCATCCGGGCCAGATCCGCT | |
| Consensus | (1251) | GGAGGTGGCAAGGCCATGTACGCCCCCCCCCATCCGGGCCAGATCCGCT | 1300 |

FIG. 66A-9

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| | | | |
|------------------------|--------|--|------|
| gp160.modSF162 | (1301) | GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG | 1350 |
| gp160.modSF162.delV2 | (1220) | GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG | |
| gp160.modSF162.delV1V2 | (1112) | GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG | |
| gp140.modSF162 | (1301) | GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG | |
| gp140.mut.modSF162 | (1301) | GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG | |
| gp140.mut7.modSF162 | (1301) | GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG | |
| gp140.mut8.modSF162 | (1301) | GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG | |
| gp120.modSF162 | (1301) | GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG | |
| Consensus | (1301) | GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG | 1400 |
| gp160.modSF162 | (1351) | ATCAGCAACACACCCGAGATCTTCCGCCCGCGCGCGGCGACATGCGCGA | |
| gp160.modSF162.delV2 | (1270) | ATCAGCAACACACCCGAGATCTTCCGCCCGCGCGCGGCGACATGCGCGA | |
| gp160.modSF162.delV1V2 | (1162) | ATCAGCAACACACCCGAGATCTTCCGCCCGCGCGCGGCGACATGCGCGA | |
| gp140.modSF162 | (1351) | ATCAGCAACACACCCGAGATCTTCCGCCCGCGCGCGGCGACATGCGCGA | |
| gp140.mut.modSF162 | (1351) | ATCAGCAACACACCCGAGATCTTCCGCCCGCGCGCGGCGACATGCGCGA | |
| gp140.mut7.modSF162 | (1351) | ATCAGCAACACACCCGAGATCTTCCGCCCGCGCGCGGCGACATGCGCGA | |
| gp140.mut8.modSF162 | (1351) | ATCAGCAACACACCCGAGATCTTCCGCCCGCGCGCGGCGACATGCGCGA | |
| gp120.modSF162 | (1351) | ATCAGCAACACACCCGAGATCTTCCGCCCGCGCGCGGCGACATGCGCGA | |
| Consensus | (1351) | ATCAGCAACACACCCGAGATCTTCCGCCCGCGCGCGGCGACATGCGCGA | 1450 |
| gp160.modSF162 | (1401) | CAACTGGCGCAGCGAGCTGTACAAGTACAAGTGGTGAAGATCGAGCCCC | |
| gp160.modSF162.delV2 | (1320) | CAACTGGCGCAGCGAGCTGTACAAGTACAAGTGGTGAAGATCGAGCCCC | |
| gp160.modSF162.delV1V2 | (1212) | CAACTGGCGCAGCGAGCTGTACAAGTACAAGTGGTGAAGATCGAGCCCC | |
| gp140.modSF162 | (1401) | CAACTGGCGCAGCGAGCTGTACAAGTACAAGTGGTGAAGATCGAGCCCC | |
| gp140.mut.modSF162 | (1401) | CAACTGGCGCAGCGAGCTGTACAAGTACAAGTGGTGAAGATCGAGCCCC | |
| gp140.mut7.modSF162 | (1401) | CAACTGGCGCAGCGAGCTGTACAAGTACAAGTGGTGAAGATCGAGCCCC | |
| gp140.mut8.modSF162 | (1401) | CAACTGGCGCAGCGAGCTGTACAAGTACAAGTGGTGAAGATCGAGCCCC | |
| gp120.modSF162 | (1401) | CAACTGGCGCAGCGAGCTGTACAAGTACAAGTGGTGAAGATCGAGCCCC | |
| Consensus | (1401) | CAACTGGCGCAGCGAGCTGTACAAGTACAAGTGGTGAAGATCGAGCCCC | |

FIG. 66A-10

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| | | | |
|------------------------|--------|--|------|
| gp160.modSF162 | (1451) | TGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG | 1500 |
| gp160.modSF162.delV2 | (1370) | TGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG | |
| gp160.modSF162.delV1V2 | (1262) | TGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG | |
| gp140.modSF162 | (1451) | TGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG | |
| gp140.mut.modSF162 | (1451) | TGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG | |
| gp140.mut7.modSF162 | (1451) | TGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG | |
| gp140.mut8.modSF162 | (1451) | TGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG | |
| gp120.modSF162 | (1451) | TGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG | |
| Consensus | (1501) | TGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG | 1550 |
| gp160.modSF162 | (1501) | CGCGCGTGACCCCTGGGCGCCATGTTCTGGGCTTCCTGGGCGCCGCGG | |
| gp160.modSF162.delV2 | (1420) | CGCGCGTGACCCCTGGGCGCCATGTTCTGGGCTTCCTGGGCGCCGCGG | |
| gp160.modSF162.delV1V2 | (1312) | CGCGCGTGACCCCTGGGCGCCATGTTCTGGGCTTCCTGGGCGCCGCGG | |
| gp140.modSF162 | (1501) | CGCGCGTGACCCCTGGGCGCCATGTTCTGGGCTTCCTGGGCGCCGCGG | |
| gp140.mut.modSF162 | (1501) | AGCGCGTGACCCCTGGGCGCCATGTTCTGGGCTTCCTGGGCGCCGCGG | |
| gp140.mut7.modSF162 | (1501) | AGCGCGTGACCCCTGGGCGCCATGTTCTGGGCTTCCTGGGCGCCGCGG | |
| gp140.mut8.modSF162 | (1501) | AGCGCGTGACCCCTGGGCGCCATGTTCTGGGCTTCCTGGGCGCCGCGG | |
| gp120.modSF162 | (1501) | CGC-----TAACTCGAG----- | |
| Consensus | (1501) | CGCGCGTGACCCCTGGGCGCCATGTTCTGGGCTTCCTGGGCGCCGCGG | 1600 |
| gp160.modSF162 | (1551) | CAGCACCATGGGCGCCCGCAGCCTGACCTGACCGTGACGGCCCGCCAGC | |
| gp160.modSF162.delV2 | (1470) | CAGCACCATGGGCGCCCGCAGCCTGACCTGACCGTGACGGCCCGCCAGC | |
| gp160.modSF162.delV1V2 | (1362) | CAGCACCATGGGCGCCCGCAGCCTGACCTGACCGTGACGGCCCGCCAGC | |
| gp140.modSF162 | (1551) | CAGCACCATGGGCGCCCGCAGCCTGACCTGACCGTGACGGCCCGCCAGC | |
| gp140.mut.modSF162 | (1551) | CAGCACCATGGGCGCCCGCAGCCTGACCTGACCGTGACGGCCCGCCAGC | |
| gp140.mut7.modSF162 | (1551) | CAGCACCATGGGCGCCCGCAGCCTGACCTGACCGTGACGGCCCGCCAGC | |
| gp140.mut8.modSF162 | (1551) | CAGCACCATGGGCGCCCGCAGCCTGACCTGACCGTGACGGCCCGCCAGC | |
| gp120.modSF162 | (1513) | ----- | |
| Consensus | (1551) | CAGCACCATGGGCGCCCGCAGCCTGACCGTGACCGTGACGGCCCGCCAGC | |

FIG. 66A-11

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| | | | |
|--------|--------|------------------------|---|
| 1601 | 1650 | | |
| | | gp160.modSF162 | TGCTGAGCGGCATCGTGCAGCAGCAGAGAACAACCTGCTGCGGCCATCGAG |
| (1601) | (1601) | gp160.modSF162.delV2 | TGCTGAGCGGCATCGTGCAGCAGCAGAGAACAACCTGCTGCGGCCATCGAG |
| (1520) | (1520) | gp160.modSF162.delV1V2 | TGCTGAGCGGCATCGTGCAGCAGCAGAGAACAACCTGCTGCGGCCATCGAG |
| (1412) | (1412) | gp140.modSF162 | TGCTGAGCGGCATCGTGCAGCAGCAGAGAACAACCTGCTGCGGCCATCGAG |
| (1601) | (1601) | gp140.modSF162 | TGCTGAGCGGCATCGTGCAGCAGCAGAGAACAACCTGCTGCGGCCATCGAG |
| (1601) | (1601) | gp140.modSF162 | TGCTGAGCGGCATCGTGCAGCAGCAGAGAACAACCTGCTGCGGCCATCGAG |
| (1601) | (1601) | gp140.modSF162 | TGCTGAGCGGCATCGTGCAGCAGCAGAGAACAACCTGCTGCGGCCATCGAG |
| (1601) | (1601) | gp140.modSF162 | TGCTGAGCGGCATCGTGCAGCAGCAGAGAACAACCTGCTGCGGCCATCGAG |
| (1513) | (1513) | gp120.modSF162 | TGCTGAGCGGCATCGTGCAGCAGCAGAGAACAACCTGCTGCGGCCATCGAG |
| (1601) | (1601) | Consensus | TGCTGAGCGGCATCGTGCAGCAGCAGAGAACAACCTGCTGCGGCCATCGAG |
| 1651 | 1700 | | |
| | | gp160.modSF162 | GCCAGCAGCACCTGCTGCAGCTGACCGTGCGGGGCATCAAGCAGCTGCA |
| (1651) | (1651) | gp160.modSF162.delV2 | GCCAGCAGCACCTGCTGCAGCTGACCGTGCGGGGCATCAAGCAGCTGCA |
| (1570) | (1570) | gp160.modSF162.delV1V2 | GCCAGCAGCACCTGCTGCAGCTGACCGTGCGGGGCATCAAGCAGCTGCA |
| (1462) | (1462) | gp140.modSF162 | GCCAGCAGCACCTGCTGCAGCTGACCGTGCGGGGCATCAAGCAGCTGCA |
| (1651) | (1651) | gp140.modSF162 | GCCAGCAGCACCTGCTGCAGCTGACCGTGCGGGGCATCAAGCAGCTGCA |
| (1651) | (1651) | gp140.modSF162 | GCCAGCAGCACCTGCTGCAGCTGACCGTGCGGGGCATCAAGCAGCTGCA |
| (1651) | (1651) | gp140.modSF162 | GCCAGCAGCACCTGCTGCAGCTGACCGTGCGGGGCATCAAGCAGCTGCA |
| (1651) | (1651) | gp140.modSF162 | GCCAGCAGCACCTGCTGCAGCTGACCGTGCGGGGCATCAAGCAGCTGCA |
| (1513) | (1513) | gp120.modSF162 | GCCAGCAGCACCTGCTGCAGCTGACCGTGCGGGGCATCAAGCAGCTGCA |
| (1651) | (1651) | Consensus | GCCAGCAGCACCTGCTGCAGCTGACCGTGCGGGGCATCAAGCAGCTGCA |
| 1701 | 1750 | | |
| | | gp160.modSF162 | GGCCCGGTGCTGGCCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG |
| (1701) | (1701) | gp160.modSF162.delV2 | GGCCCGGTGCTGGCCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG |
| (1620) | (1620) | gp160.modSF162.delV1V2 | GGCCCGGTGCTGGCCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG |
| (1512) | (1512) | gp140.modSF162 | GGCCCGGTGCTGGCCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG |
| (1701) | (1701) | gp140.modSF162 | GGCCCGGTGCTGGCCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG |
| (1701) | (1701) | gp140.modSF162 | GGCCCGGTGCTGGCCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG |
| (1701) | (1701) | gp140.modSF162 | GGCCCGGTGCTGGCCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG |
| (1701) | (1701) | gp140.modSF162 | GGCCCGGTGCTGGCCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG |
| (1701) | (1701) | gp140.modSF162 | GGCCCGGTGCTGGCCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG |

FIG. 66A-12

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| | | |
|------------------------|--------|---|
| gp120.modSF162 | (1513) | ----- |
| Consensus | (1701) | GGCCCGCTGCTGGCCCGTGAGCGCTACCTGAAGGACCAGCAGCTGCTGG |
| | | 1800 |
| gp160.modSF162 | (1751) | GCATCTGGGGTGCAGCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGG |
| gp160.modSF162.delV2 | (1670) | GCATCTGGGGTGCAGCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGG |
| gp160.modSF162.delV1V2 | (1562) | GCATCTGGGGTGCAGCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGG |
| gp140.modSF162 | (1751) | GCATCTGGGGTGCAGCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGG |
| gp140.mut.modSF162 | (1751) | GCATCTGGGGTGCAGCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGG |
| gp140.mut7.modSF162 | (1751) | GCATCTGGGGTGCAGCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGG |
| gp140.mut8.modSF162 | (1751) | GCATCTGGGGTGCAGCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGG |
| gp120.modSF162 | (1513) | ----- |
| Consensus | (1751) | GCATCTGGGGTGCAGCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGG |
| | | 1801 |
| gp160.modSF162 | (1801) | AACGCCAGCTGGAGCAACAAGAGCCCTGGACCAGATCTGGAACAACATGAC |
| gp160.modSF162.delV2 | (1720) | AACGCCAGCTGGAGCAACAAGAGCCCTGGACCAGATCTGGAACAACATGAC |
| gp160.modSF162.delV1V2 | (1612) | AACGCCAGCTGGAGCAACAAGAGCCCTGGACCAGATCTGGAACAACATGAC |
| gp140.modSF162 | (1801) | AACGCCAGCTGGAGCAACAAGAGCCCTGGACCAGATCTGGAACAACATGAC |
| gp140.mut.modSF162 | (1801) | AACGCCAGCTGGAGCAACAAGAGCCCTGGACCAGATCTGGAACAACATGAC |
| gp140.mut7.modSF162 | (1801) | AACGCCAGCTGGAGCAACAAGAGCCCTGGACCAGATCTGGAACAACATGAC |
| gp140.mut8.modSF162 | (1801) | AACGCCAGCTGGAGCAACAAGAGCCCTGGACCAGATCTGGAACAACATGAC |
| gp120.modSF162 | (1513) | ----- |
| Consensus | (1801) | AACGCCAGCTGGAGCAACAAGAGCCCTGGACCAGATCTGGAACAACATGAC |
| | | 1851 |
| gp160.modSF162 | (1851) | CTGGATGGAGTGGGAGCGCGAGATCGACAACCTACACCAACCTGATCTACA |
| gp160.modSF162.delV2 | (1770) | CTGGATGGAGTGGGAGCGCGAGATCGACAACCTACACCAACCTGATCTACA |
| gp160.modSF162.delV1V2 | (1662) | CTGGATGGAGTGGGAGCGCGAGATCGACAACCTACACCAACCTGATCTACA |
| gp140.modSF162 | (1851) | CTGGATGGAGTGGGAGCGCGAGATCGACAACCTACACCAACCTGATCTACA |
| gp140.mut.modSF162 | (1851) | CTGGATGGAGTGGGAGCGCGAGATCGACAACCTACACCAACCTGATCTACA |
| gp140.mut7.modSF162 | (1851) | CTGGATGGAGTGGGAGCGCGAGATCGACAACCTACACCAACCTGATCTACA |
| gp140.mut8.modSF162 | (1851) | CTGGATGGAGTGGGAGCGCGAGATCGACAACCTACACCAACCTGATCTACA |
| gp120.modSF162 | (1513) | ----- |
| Consensus | (1851) | CTGGATGGAGTGGGAGCGCGAGATCGACAACCTACACCAACCTGATCTACA |

FIG. 66A-13

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| | | | |
|------------------------|--------|--|------|
| | | | 1950 |
| | | | 1951 |
| gp160.modSF162 | (1901) | CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGGAGAACGACGAGGAGCTG | |
| gp160.modSF162.delV2 | (1820) | CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGGAGAACGACGAGGAGCTG | |
| gp160.modSF162.delV1V2 | (1712) | CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGGAGAACGACGAGGAGCTG | |
| gp140.modSF162 | (1901) | CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGGAGAACGACGAGGAGCTG | |
| gp140.mut.modSF162 | (1901) | CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGGAGAACGACGAGGAGCTG | |
| gp140.mut7.modSF162 | (1901) | CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGGAGAACGACGAGGAGCTG | |
| gp140.mut8.modSF162 | (1901) | CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGGAGAACGACGAGGAGCTG | |
| gp120.modSF162 | (1513) | ----- | |
| Consensus | (1901) | CCCTGATCGAGGAGAGCCAGAACCCAGCAGGAGGAGAACGACGAGGAGCTG | |
| | | | 2000 |
| | | | 1951 |
| gp160.modSF162 | (1951) | CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAA | |
| gp160.modSF162.delV2 | (1870) | CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAA | |
| gp160.modSF162.delV1V2 | (1762) | CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAA | |
| gp140.modSF162 | (1951) | CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAA | |
| gp140.mut.modSF162 | (1951) | CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAA | |
| gp140.mut7.modSF162 | (1951) | CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAA | |
| gp140.mut8.modSF162 | (1951) | CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAA | |
| gp120.modSF162 | (1513) | ----- | |
| Consensus | (1951) | CTGGAGCTGGACAAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAA | |
| | | | 2050 |
| gp160.modSF162 | (2001) | GTGGCTGTGGTACATCAAGATCTTCATCATGATCGTGGCGGCCCTGGTGG | |
| gp160.modSF162.delV2 | (1920) | GTGGCTGTGGTACATCAAGATCTTCATCATGATCGTGGCGGCCCTGGTGG | |
| gp160.modSF162.delV1V2 | (1812) | GTGGCTGTGGTACATCAAGATCTTCATCATGATCGTGGCGGCCCTGGTGG | |
| gp140.modSF162 | (2001) | GTGGCTGTGGTACATCTAACTCGAG----- | |
| gp140.mut.modSF162 | (2001) | GTGGCTGTGGTACATCTAACTCGAG----- | |

FIG. 66A-14

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| | | | |
|------------------------|--------|--|------|
| gp140.mut7.modSF162 | (2001) | GTGGCTGTGTACATCTAACTCGAG | 2100 |
| gp140.mut8.modSF162 | (2001) | GTGGCTGTGTACATCTAACTCGAG | |
| gp120.modSF162 | (1513) | | |
| Consensus | (2001) | GTGGCTGTGTACATCTAACTCGAG | |
| gp160.modSF162 | (2051) | GCCTGGGCATCGTGTTCACCGTGTGAGCATCGTGAACCGGTGCGCCAG | |
| gp160.modSF162.delV2 | (1970) | GCCTGGGCATCGTGTTCACCGTGTGAGCATCGTGAACCGGTGCGCCAG | |
| gp160.modSF162.delV1V2 | (1862) | GCCTGGGCATCGTGTTCACCGTGTGAGCATCGTGAACCGGTGCGCCAG | |
| gp140.modSF162 | (2026) | | |
| gp140.mut.modSF162 | (2026) | | |
| gp140.mut7.modSF162 | (2026) | | |
| gp140.mut8.modSF162 | (2026) | | |
| gp120.modSF162 | (1513) | | |
| Consensus | (2051) | | |
| gp160.modSF162 | (2101) | GGCTACAGCCCTGAGCTTCCAGACCCGCTTCCCCGCCCCCGCGGCC | 2150 |
| gp160.modSF162.delV2 | (2020) | GGCTACAGCCCTGAGCTTCCAGACCCGCTTCCCCGCCCCCGCGGCC | |
| gp160.modSF162.delV1V2 | (1912) | GGCTACAGCCCTGAGCTTCCAGACCCGCTTCCCCGCCCCCGCGGCC | |
| gp140.modSF162 | (2026) | | |
| gp140.mut.modSF162 | (2026) | | |
| gp140.mut7.modSF162 | (2026) | | |
| gp140.mut8.modSF162 | (2026) | | |
| gp120.modSF162 | (1513) | | |
| Consensus | (2101) | | |
| gp160.modSF162 | (2151) | CGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGGACCGCGACC | 2200 |
| gp160.modSF162.delV2 | (2070) | CGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGGACCGCGACC | |
| gp160.modSF162.delV1V2 | (1962) | CGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGGACCGCGACC | |
| gp140.modSF162 | (2026) | | |
| gp140.mut.modSF162 | (2026) | | |
| gp140.mut7.modSF162 | (2026) | | |
| gp140.mut8.modSF162 | (2026) | | |
| gp120.modSF162 | (1513) | | |
| Consensus | (2151) | | |

FIG. 66A-15

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| | | | | |
|------------------------|--------|------|---|------|
| gp160.modSF162 | (2201) | 2201 | GCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGGGACGACCTG | 2250 |
| gp160.modSF162.delV2 | (2120) | | GCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGGGACGACCTG | |
| gp160.modSF162.delV1V2 | (2012) | | GCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGGGACGACCTG | |
| gp140.modSF162 | (2026) | | ----- | |
| gp140.modSF162 | (2026) | | ----- | |
| gp140.mut.modSF162 | (2026) | | ----- | |
| gp140.mut7.modSF162 | (2026) | | ----- | |
| gp140.mut8.modSF162 | (2026) | | ----- | |
| gp120.modSF162 | (1513) | | ----- | |
| Consensus | (2201) | | ----- | |
| gp160.modSF162 | (2251) | 2251 | CGCAGCCTGTGCCTGTTCAAGTACCAACCGCCTGCGCGACCTGATCCTGAT | 2300 |
| gp160.modSF162.delV2 | (2170) | | CGCAGCCTGTGCCTGTTCAAGTACCAACCGCCTGCGCGACCTGATCCTGAT | |
| gp160.modSF162.delV1V2 | (2062) | | CGCAGCCTGTGCCTGTTCAAGTACCAACCGCCTGCGCGACCTGATCCTGAT | |
| gp140.modSF162 | (2026) | | ----- | |
| gp140.mut.modSF162 | (2026) | | ----- | |
| gp140.mut7.modSF162 | (2026) | | ----- | |
| gp140.mut8.modSF162 | (2026) | | ----- | |
| gp120.modSF162 | (1513) | | ----- | |
| Consensus | (2251) | | ----- | |
| gp160.modSF162 | (2301) | 2301 | CGCCGCCCGCATCGTGGAGCTGCTGGGCCCGCCGGCTGGGAGGCCCTGA | 2350 |
| gp160.modSF162.delV2 | (2220) | | CGCCGCCCGCATCGTGGAGCTGCTGGGCCCGCCGGCTGGGAGGCCCTGA | |
| gp160.modSF162.delV1V2 | (2112) | | CGCCGCCCGCATCGTGGAGCTGCTGGGCCCGCCGGCTGGGAGGCCCTGA | |

FIG. 66A-16

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| | | | |
|------------------------|--------|---|------|
| gp140.modSF162 | (2026) | 2351 | 2400 |
| gp140.mut.modSF162 | (2026) | AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC | |
| gp140.mut7.modSF162 | (2026) | AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC | |
| gp140.mut8.modSF162 | (2026) | AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC | |
| gp120.modSF162 | (1513) | ----- | |
| Consensus | (2301) | ----- | |
| gp160.modSF162 | (2351) | AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC | |
| gp160.modSF162.delV2 | (2270) | AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC | |
| gp160.modSF162.delV1V2 | (2162) | AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC | |
| gp140.modSF162 | (2026) | ----- | |
| gp140.mut.modSF162 | (2026) | ----- | |
| gp140.mut7.modSF162 | (2026) | ----- | |
| gp140.mut8.modSF162 | (2026) | ----- | |
| gp120.modSF162 | (1513) | ----- | |
| Consensus | (2351) | ----- | |
| gp160.modSF162 | (2401) | 2450 | |
| gp160.modSF162.delV2 | (2320) | GCCGTGAGCCTGTTTCGACGCCATCGCCATCGCCGTGGCCGAGGGCACCGA | |
| gp160.modSF162.delV1V2 | (2212) | GCCGTGAGCCTGTTTCGACGCCATCGCCATCGCCGTGGCCGAGGGCACCGA | |
| gp140.modSF162 | (2026) | GCCGTGAGCCTGTTTCGACGCCATCGCCATCGCCGTGGCCGAGGGCACCGA | |
| gp140.mut.modSF162 | (2026) | ----- | |
| gp140.mut7.modSF162 | (2026) | ----- | |
| gp140.mut8.modSF162 | (2026) | ----- | |
| gp120.modSF162 | (1513) | ----- | |
| Consensus | (2401) | ----- | |

FIG. 66A-17

SUBSTITUTE SHEET (RULE 26)

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| | | | |
|------------------------|--------|---|------|
| gp160.modSF162 | (2451) | 2451 | 2500 |
| gp160.modSF162.delV2 | (2370) | CCGCATCATCGAGGTGGCCCGAGCGCATCGGCCGCGCCTTCCTGCACATCC | |
| gp160.modSF162.delV1V2 | (2262) | CCGCATCATCGAGGTGGCCCGAGCGCATCGGCCGCGCCTTCCTGCACATCC | |
| gp140.modSF162 | (2026) | CCGCATCATCGAGGTGGCCCGAGCGCATCGGCCGCGCCTTCCTGCACATCC | |
| gp140.mut.modSF162 | (2026) | ----- | |
| gp140.mut7.modSF162 | (2026) | ----- | |
| gp140.mut8.modSF162 | (2026) | ----- | |
| gp120.modSF162 | (1513) | ----- | |
| Consensus | (2451) | ----- | |
| gp160.modSF162 | (2501) | 2501 | 2547 |
| gp160.modSF162.delV2 | (2420) | CCCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCTGTAACTCGAG | |
| gp160.modSF162.delV1V2 | (2312) | CCCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCTGTAACTCGAG | |
| gp140.modSF162 | (2026) | ----- | |
| gp140.mut.modSF162 | (2026) | ----- | |
| gp140.mut7.modSF162 | (2026) | ----- | |
| gp140.mut8.modSF162 | (2026) | ----- | |
| gp120.modSF162 | (1513) | ----- | |
| Consensus | (2501) | ----- | |

FIG. 66A-18

SUBSTITUTE SHEET (RULE 26)

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| | | | | |
|--------------------|------------|---|--------------|-----|
| | | | Start of tPA | |
| | 1 | | ↓ | 40 |
| gp160 | (1) | GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT | | |
| gp160 del V1 | (1) | GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT | | |
| gp160 del V2 | (1) | GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT | | |
| gp160 del V1-2 | (1) | GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT | | |
| gp 160 del 128-194 | (1) | GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT | | |
| gp140TM | (1) | GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT | | |
| gp140 | (1) | GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT | | |
| gp140mut | (1) | GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT | | |
| gp120 | (1) | GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT | | |
| Consensus | (1) | GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT | | |
| | 41 | | | 80 |
| gp160 | (41) | GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCCAG | | |
| gp160 del V1 | (41) | GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCCAG | | |
| gp160 del V2 | (41) | GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCCAG | | |
| gp160 del V1-2 | (41) | GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCCAG | | |
| gp 160 del 128-194 | (41) | GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCCAG | | |
| gp140TM | (41) | GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCCAG | | |
| gp140 | (41) | GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCCAG | | |
| gp140mut | (41) | GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCCAG | | |
| gp120 | (41) | GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCCAG | | |
| Consensus | (41) | GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCCAG | | |
| | end of tPA | | ↓ | 120 |
| gp160 | (81) | CGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG | | |
| gp160 del V1 | (81) | CGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG | | |
| gp160 del V2 | (81) | CGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG | | |
| gp160 del V1-2 | (81) | CGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG | | |
| gp 160 del 128-194 | (81) | CGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG | | |
| gp140TM | (81) | CGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG | | |
| gp140 | (81) | CGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG | | |
| gp140mut | (81) | CGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG | | |
| gp120 | (81) | CGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG | | |
| Consensus | (81) | CGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG | | |
| | 121 | | | 160 |
| gp 160 | (121) | CCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA | | |
| gp160 del V1 | (121) | CCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA | | |
| gp160 del V2 | (121) | CCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA | | |
| gp160 del V1-2 | (121) | CCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA | | |
| gp 160 del 128-194 | (121) | CCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA | | |
| gp140TM | (121) | CCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA | | |
| gp140 | (121) | CCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA | | |
| gp140mut | (121) | CCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA | | |
| gp120 | (121) | CCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA | | |
| Consensus | (121) | CCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCA | | |

FIG. 66B-1

SUBSTITUTE SHEET (RULE 26)

| 98 / 131 | | 161 | 200 |
|--------------------|-------|--|-----|
| gp160 | (161) | GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAGTGTG | |
| gp160 del V1 | (161) | GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAGTGTG | |
| gp160 del V2 | (161) | GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAGTGTG | |
| gp160 del V1-2 | (161) | GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAGTGTG | |
| gp 160 del 128-194 | (161) | GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAGTGTG | |
| gp140TM | (161) | GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAGTGTG | |
| gp140 | (161) | GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAGTGTG | |
| gp140mut | (161) | GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAGTGTG | |
| gp120 | (161) | GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAGTGTG | |
| Consensus | (161) | GCGACGCCAAGGCTTACAAGGCCGAGGCCCAACAGTGTG | |
| | | 201 | 240 |
| gp160 | (201) | GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG | |
| gp160 del V1 | (201) | GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG | |
| gp160 del V2 | (201) | GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG | |
| gp160 del V1-2 | (201) | GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG | |
| gp 160 del 128-194 | (201) | GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG | |
| gp140TM | (201) | GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG | |
| gp140 | (201) | GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG | |
| gp140mut | (201) | GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG | |
| gp120 | (201) | GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG | |
| Consensus | (201) | GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG | |
| | | 241 | 280 |
| gp160 | (241) | GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT | |
| gp160 del V1 | (241) | GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT | |
| gp160 del V2 | (241) | GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT | |
| gp160 del V1-2 | (241) | GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT | |
| gp 160 del 128-194 | (241) | GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT | |
| gp140TM | (241) | GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT | |
| gp140 | (241) | GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT | |
| gp140mut | (241) | GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT | |
| gp120 | (241) | GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT | |
| Consensus | (241) | GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT | |
| | | 281 | 320 |
| gp160 | (281) | GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT | |
| gp160 del V1 | (281) | GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT | |
| gp160 del V2 | (281) | GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT | |
| gp160 del V1-2 | (281) | GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT | |
| gp 160 del 128-194 | (281) | GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT | |
| gp140TM | (281) | GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT | |
| gp140 | (281) | GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT | |
| gp140mut | (281) | GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT | |
| gp120 | (281) | GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT | |
| Consensus | (281) | GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT | |
| | | 321 | 360 |
| gp160 | (321) | CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG | |
| gp160 del V1 | (321) | CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG | |
| gp160 del V2 | (321) | CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG | |
| gp160 del V1-2 | (321) | CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG | |
| gp 160 del 128-194 | (321) | CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG | |
| gp140TM | (321) | CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG | |
| gp140 | (321) | CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG | |
| gp140mut | (321) | CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG | |
| gp120 | (321) | CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG | |
| Consensus | (321) | CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG | |

FIG. 66B-2

SUBSTITUTE SHEET (RULE 26)

| | | |
|--------------------|---|-----|
| | 99/131 | 400 |
| | 361 | |
| gp160 | (361) ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA | |
| gp160 del V1 | (361) ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGG | |
| gp160 del V2 | (361) ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA | |
| gp160 del V1-2 | (361) GGC----- | |
| gp 160 del 128-194 | (361) ACCCCCCTGTGCGTGCGGGGCGAGG----- | |
| gp140TM | (361) ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA | |
| gp140 | (361) ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA | |
| gp140mut | (361) ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA | |
| gp120 | (361) ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA | |
| Consensus | (361) ACCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGA | 440 |
| | 401 | |
| gp160 | (401) CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA | |
| gp160 del V1 | (401) GCGCCGGC----- | |
| gp160 del V2 | (401) CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA | |
| gp160 del V1-2 | (364) ----- | |
| gp 160 del 128-194 | (385) ----- | |
| gp140TM | (401) CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA | |
| gp140 | (401) CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA | |
| gp140mut | (401) CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA | |
| gp120 | (401) CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA | |
| Consensus | (401) CCGGCAGCACCAACGGCACCAACAGCACCAGCGGCACCAA | 480 |
| | 441 | |
| gp160 | (441) CAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACC | |
| gp160 del V1 | (409) ----- | |
| gp160 del V2 | (441) CAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACC | |
| gp160 del V1-2 | (364) ----- | |
| gp 160 del 128-194 | (385) ----- | |
| gp140TM | (441) CAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACC | |
| gp140 | (441) CAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACC | |
| gp140mut | (441) CAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACC | |
| gp120 | (441) CAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACC | |
| Consensus | (441) CAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACC | 520 |
| | 481 | |
| gp160 | (481) GACAGCTGGGAGAAGATGCCCCGAGGGCGAGATCAAGAACT | |
| gp160 del V1 | (409) -----GGCGAGATCAAGAACT | |
| gp160 del V2 | (481) GACAGCTGGGAGAAGATGCCCCGAGGGCGAGATCAAGAACT | |
| gp160 del V1-2 | (364) ----- | |
| gp 160 del 128-194 | (385) ----- | |
| gp140TM | (481) GACAGCTGGGAGAAGATGCCCCGAGGGCGAGATCAAGAACT | |
| gp140 | (481) GACAGCTGGGAGAAGATGCCCCGAGGGCGAGATCAAGAACT | |
| gp140mut | (481) GACAGCTGGGAGAAGATGCCCCGAGGGCGAGATCAAGAACT | |
| gp120 | (481) GACAGCTGGGAGAAGATGCCCCGAGGGCGAGATCAAGAACT | |
| Consensus | (481) GACAGCTGGGAGAAGATGCCCCGAGGGCGAGATCAAGAACT | 560 |
| | 521 | |
| gp160 | (521) GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA | |
| gp160 del V1 | (521) GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA | |
| gp160 del V2 | (521) GCAGCTTCAACATCGGCGCCGCG----- | |
| gp160 del V1-2 | (521) ----- | |
| gp 160 del 128-194 | (521) ----- | |
| gp140TM | (521) GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA | |
| gp140 | (521) GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA | |
| gp140mut | (521) GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA | |
| gp120 | (521) GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA | |
| Consensus | (521) GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA | |

FIG. 66B-3

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| | | | | |
|--------------------|-------|--|--|-----|
| | | 561 | | 600 |
| gp160 | (561) | GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC | | |
| gp160 del V1 | (465) | GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC | | |
| gp160 del V2 | (544) | ----- | | |
| gp160 del V1-2 | (364) | ----- | | |
| gp 160 del 128-194 | (385) | ----- | | |
| gp140TM | (561) | GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC | | |
| gp140 | (561) | GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC | | |
| gp140mut | (561) | GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC | | |
| gp120 | (561) | GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC | | |
| Consensus | (561) | GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC | | 640 |
| | | 601 | | |
| gp160 | (601) | ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA | | |
| gp160 del V1 | (505) | ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA | | |
| gp160 del V2 | (544) | -----CGCCTGATCAACTGCA | | |
| gp160 del V1-2 | (364) | ----- | | |
| gp 160 del 128-194 | (385) | -----AACTGCG | | |
| gp140TM | (601) | ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA | | |
| gp140 | (601) | ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA | | |
| gp140mut | (601) | ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA | | |
| gp120 | (601) | ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA | | |
| Consensus | (601) | ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA | | 680 |
| | | 641 | | |
| gp160 | (641) | ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT | | |
| gp160 del V1 | (545) | ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT | | |
| gp160 del V2 | (560) | ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT | | |
| gp160 del V1-2 | (364) | -----CAGGCCTGCCCCAAGGTGAGCTT | | |
| gp 160 del 128-194 | (392) | AGACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT | | |
| gp140TM | (641) | ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT | | |
| gp140 | (641) | ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT | | |
| gp140mut | (641) | ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT | | |
| gp120 | (641) | ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT | | |
| Consensus | (641) | ACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTT | | 720 |
| | | 681 | | |
| gp160 | (681) | CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC | | |
| gp160 del V1 | (585) | CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC | | |
| gp160 del V2 | (600) | CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC | | |
| gp160 del V1-2 | (387) | CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC | | |
| gp 160 del 128-194 | (432) | CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC | | |
| gp140TM | (681) | CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC | | |
| gp140 | (681) | CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC | | |
| gp140mut | (681) | CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC | | |
| gp120 | (681) | CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC | | |
| Consensus | (681) | CGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC | | 760 |
| | | 721 | | |
| gp160 | (721) | GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG | | |
| gp160 del V1 | (625) | GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG | | |
| gp160 del V2 | (640) | GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG | | |
| gp160 del V1-2 | (427) | GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG | | |
| gp 160 del 128-194 | (472) | GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG | | |
| gp140TM | (721) | GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG | | |
| gp140 | (721) | GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG | | |
| gp140mut | (721) | GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG | | |
| gp120 | (721) | GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG | | |
| Consensus | (721) | GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG | | |

FIG. 66B-4

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| | | | |
|--------------------|-------|--|-----|
| | | 761 | 800 |
| gp160 | (761) | GCCCCTGCAAGAACGTGAGCACCGTGAGTGCACCCACGG | |
| gp160 del V1 | (665) | GCCCCTGCAAGAACGTGAGCACCGTGAGTGCACCCACGG | |
| gp160 del V2 | (680) | GCCCCTGCAAGAACGTGAGCACCGTGAGTGCACCCACGG | |
| gp160 del V1-2 | (467) | GCCCCTGCAAGAACGTGAGCACCGTGAGTGCACCCACGG | |
| gp 160 del 128-194 | (512) | GCCCCTGCAAGAACGTGAGCACCGTGAGTGCACCCACGG | |
| gp140TM | (761) | GCCCCTGCAAGAACGTGAGCACCGTGAGTGCACCCACGG | |
| gp140 | (761) | GCCCCTGCAAGAACGTGAGCACCGTGAGTGCACCCACGG | |
| gp140mut | (761) | GCCCCTGCAAGAACGTGAGCACCGTGAGTGCACCCACGG | |
| gp120 | (761) | GCCCCTGCAAGAACGTGAGCACCGTGAGTGCACCCACGG | |
| Consensus | (761) | GCCCCTGCAAGAACGTGAGCACCGTGAGTGCACCCACGG | 840 |
| | | 801 | |
| gp160 | (801) | CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC | |
| gp160 del V1 | (705) | CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC | |
| gp160 del V2 | (720) | CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC | |
| gp160 del V1-2 | (507) | CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC | |
| gp 160 del 128-194 | (552) | CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC | |
| gp140TM | (801) | CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC | |
| gp140 | (801) | CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC | |
| gp140mut | (801) | CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC | |
| gp120 | (801) | CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC | |
| Consensus | (801) | CATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC | 880 |
| | | 841 | |
| gp160 | (841) | AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT | |
| gp160 del V1 | (745) | AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT | |
| gp160 del V2 | (760) | AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT | |
| gp160 del V1-2 | (547) | AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT | |
| gp 160 del 128-194 | (592) | AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT | |
| gp140TM | (841) | AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT | |
| gp140 | (841) | AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT | |
| gp140mut | (841) | AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT | |
| gp120 | (841) | AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT | |
| Consensus | (841) | AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT | 920 |
| | | 881 | |
| gp160 | (881) | TCACCGACAACGCCAAGACCATCATCGTGCAGCTGAACGA | |
| gp160 del V1 | (785) | TCACCGACAACGCCAAGACCATCATCGTGCAGCTGAACGA | |
| gp160 del V2 | (800) | TCACCGACAACGCCAAGACCATCATCGTGCAGCTGAACGA | |
| gp160 del V1-2 | (587) | TCACCGACAACGCCAAGACCATCATCGTGCAGCTGAACGA | |
| gp 160 del 128-194 | (632) | TCACCGACAACGCCAAGACCATCATCGTGCAGCTGAACGA | |
| gp140TM | (881) | TCACCGACAACGCCAAGACCATCATCGTGCAGCTGAACGA | |
| gp140 | (881) | TCACCGACAACGCCAAGACCATCATCGTGCAGCTGAACGA | |
| gp140mut | (881) | TCACCGACAACGCCAAGACCATCATCGTGCAGCTGAACGA | |
| gp120 | (881) | TCACCGACAACGCCAAGACCATCATCGTGCAGCTGAACGA | |
| Consensus | (881) | TCACCGACAACGCCAAGACCATCATCGTGCAGCTGAACGA | 960 |
| | | 921 | |
| gp160 | (921) | GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG | |
| gp160 del V1 | (825) | GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG | |
| gp160 del V2 | (840) | GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG | |
| gp160 del V1-2 | (627) | GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG | |
| gp 160 del 128-194 | (672) | GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG | |
| gp140TM | (921) | GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG | |
| gp140 | (921) | GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG | |
| gp140mut | (921) | GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG | |
| gp120 | (921) | GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG | |
| Consensus | (921) | GTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG | |

FIG. 66B-5

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| | | |
|--------------------|--|------|
| | 961 | 1000 |
| gp160 | (961) CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG | |
| gp160 del V1 | (865) CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG | |
| gp160 del V2 | (880) CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG | |
| gp160 del V1-2 | (667) CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG | |
| gp 160 del 128-194 | (712) CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG | |
| gp140TM | (961) CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG | |
| gp140 | (961) CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG | |
| gp140mut | (961) CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG | |
| gp120 | (961) CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG | |
| Consensus | (961) CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACG | |
| | 1001 | 1040 |
| gp160 | (1001) CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTG | |
| gp160 del V1 | (905) CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTG | |
| gp160 del V2 | (920) CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTG | |
| gp160 del V1-2 | (707) CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTG | |
| gp 160 del 128-194 | (752) CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTG | |
| gp140TM | (1001) CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTG | |
| gp140 | (1001) CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTG | |
| gp140mut | (1001) CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTG | |
| gp120 | (1001) CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTG | |
| Consensus | (1001) CCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTG | |
| | 1041 | 1080 |
| gp160 | (1041) CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG | |
| gp160 del V1 | (945) CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG | |
| gp160 del V2 | (960) CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG | |
| gp160 del V1-2 | (747) CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG | |
| gp 160 del 128-194 | (792) CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG | |
| gp140TM | (1041) CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG | |
| gp140 | (1041) CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG | |
| gp140mut | (1041) CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG | |
| gp120 | (1041) CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG | |
| Consensus | (1041) CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG | |
| | 1081 | 1120 |
| gp160 | (1081) ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA | |
| gp160 del V1 | (985) ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA | |
| gp160 del V2 | (1000) ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA | |
| gp160 del V1-2 | (787) ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA | |
| gp 160 del 128-194 | (832) ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA | |
| gp140TM | (1081) ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA | |
| gp140 | (1081) ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA | |
| gp140mut | (1081) ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA | |
| gp120 | (1081) ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA | |
| Consensus | (1081) ATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGA | |
| | 1121 | 1160 |
| gp160 | (1121) CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT | |
| gp160 del V1 | (1025) CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT | |
| gp160 del V2 | (1040) CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT | |
| gp160 del V1-2 | (827) CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT | |
| gp 160 del 128-194 | (872) CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT | |
| gp140TM | (1121) CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT | |
| gp140 | (1121) CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT | |
| gp140mut | (1121) CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT | |
| gp120 | (1121) CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT | |
| Consensus | (1121) CCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGAT | |

FIG. 66B-6

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| | | | |
|--------------------|--------|--|------|
| | | | 1200 |
| | | 1161 | |
| gp160 | (1161) | CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC | |
| gp160 del V1 | (1065) | CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC | |
| gp160 del V2 | (1080) | CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC | |
| gp160 del V1-2 | (867) | CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC | |
| gp 160 del 128-194 | (912) | CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC | |
| gp140TM | (1161) | CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC | |
| gp140 | (1161) | CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC | |
| gp140mut | (1161) | CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC | |
| gp120 | (1161) | CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC | |
| Consensus | (1161) | CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC | |
| | | 1201 | |
| gp160 | (1201) | TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA | |
| gp160 del V1 | (1105) | TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA | |
| gp160 del V2 | (1120) | TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA | |
| gp160 del V1-2 | (907) | TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA | |
| gp 160 del 128-194 | (952) | TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA | |
| gp140TM | (1201) | TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA | |
| gp140 | (1201) | TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA | |
| gp140mut | (1201) | TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA | |
| gp120 | (1201) | TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA | |
| Consensus | (1201) | TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA | |
| | | 1241 | |
| gp160 | (1241) | CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT | |
| gp160 del V1 | (1145) | CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT | |
| gp160 del V2 | (1160) | CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT | |
| gp160 del V1-2 | (947) | CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT | |
| gp 160 del 128-194 | (992) | CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT | |
| gp140TM | (1241) | CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT | |
| gp140 | (1241) | CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT | |
| gp140mut | (1241) | CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT | |
| gp120 | (1241) | CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT | |
| Consensus | (1241) | CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT | |
| | | 1281 | |
| gp160 | (1281) | CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG | |
| gp160 del V1 | (1185) | CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG | |
| gp160 del V2 | (1200) | CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG | |
| gp160 del V1-2 | (987) | CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG | |
| gp 160 del 128-194 | (1032) | CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG | |
| gp140TM | (1281) | CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG | |
| gp140 | (1281) | CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG | |
| gp140mut | (1281) | CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG | |
| gp120 | (1281) | CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG | |
| Consensus | (1281) | CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG | |
| | | 1321 | |
| gp160 | (1321) | GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCC | |
| gp160 del V1 | (1225) | GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCC | |
| gp160 del V2 | (1240) | GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCC | |
| gp160 del V1-2 | (1027) | GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCC | |
| gp 160 del 128-194 | (1072) | GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCC | |
| gp140TM | (1321) | GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCC | |
| gp140 | (1321) | GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCC | |
| gp140mut | (1321) | GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCC | |
| gp120 | (1321) | GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCC | |
| Consensus | (1321) | GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCC | |

FIG. 66B-7

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| | | | |
|--------------------|--------|--|------|
| | | 1361 | 1400 |
| gp160 | (1361) | AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC | |
| gp160 del V1 | (1265) | AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC | |
| gp160 del V2 | (1280) | AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC | |
| gp160 del V1-2 | (1067) | AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC | |
| gp 160 del 128-194 | (1112) | AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC | |
| gp140TM | (1361) | AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC | |
| gp140 | (1361) | AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC | |
| gp140mut | (1361) | AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC | |
| gp120 | (1361) | AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC | |
| Consensus | (1361) | AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC | |
| | | 1401 | 1440 |
| gp160 | (1401) | CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC | |
| gp160 del V1 | (1305) | CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC | |
| gp160 del V2 | (1320) | CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC | |
| gp160 del V1-2 | (1107) | CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC | |
| gp 160 del 128-194 | (1152) | CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC | |
| gp140TM | (1401) | CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC | |
| gp140 | (1401) | CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC | |
| gp140mut | (1401) | CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC | |
| gp120 | (1401) | CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC | |
| Consensus | (1401) | CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC | |
| | | 1441 | 1480 |
| gp160 | (1441) | GAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAAC | |
| gp160 del V1 | (1345) | GAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAAC | |
| gp160 del V2 | (1360) | GAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAAC | |
| gp160 del V1-2 | (1147) | GAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAAC | |
| gp 160 del 128-194 | (1192) | GAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAAC | |
| gp140TM | (1441) | GAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAAC | |
| gp140 | (1441) | GAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAAC | |
| gp140mut | (1441) | GAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAAC | |
| gp120 | (1441) | GAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAAC | |
| Consensus | (1441) | GAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAAC | |
| | | 1481 | 1520 |
| gp160 | (1481) | GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA | |
| gp160 del V1 | (1385) | GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA | |
| gp160 del V2 | (1400) | GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA | |
| gp160 del V1-2 | (1187) | GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA | |
| gp 160 del 128-194 | (1232) | GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA | |
| gp140TM | (1481) | GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA | |
| gp140 | (1481) | GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA | |
| gp140mut | (1481) | GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA | |
| gp120 | (1481) | GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA | |
| Consensus | (1481) | GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA | |
| | | 1521 | 1560 |
| gp160 | (1521) | GCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTG | |
| gp160 del V1 | (1425) | GCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTG | |
| gp160 del V2 | (1440) | GCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTG | |
| gp160 del V1-2 | (1227) | GCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTG | |
| gp 160 del 128-194 | (1272) | GCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTG | |
| gp140TM | (1521) | GCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTG | |
| gp140 | (1521) | GCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTG | |
| gp140mut | (1521) | GCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTG | |
| gp120 | (1521) | GCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTG | |
| Consensus | (1521) | GCCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGCCGCGTG | |

FIG. 66B-8

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| | | | | |
|--------------------|--------|------|--|------|
| | | 1561 | | 1600 |
| | | | GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT | |
| gp160 | (1561) | | GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT | |
| gp160 del V1 | (1465) | | GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT | |
| gp160 del V2 | (1480) | | GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT | |
| gp160 del V1-2 | (1267) | | GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT | |
| gp 160 del 128-194 | (1312) | | GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT | |
| gp140TM | (1561) | | GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT | |
| gp140 | (1561) | | GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT | |
| gp140mut | (1561) | | GTGCAGCGCGAGAAGAGCGCCGTGGGCCTGGGCGCCCTGT | |
| gp120 | (1561) | | GTGCAGCGCGAGAAGCGCTAAG----- | |
| Consensus | (1561) | | GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGT | |
| | | 1601 | | 1640 |
| gp160 | (1601) | | TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG | |
| gp160 del V1 | (1505) | | TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG | |
| gp160 del V2 | (1520) | | TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG | |
| gp160 del V1-2 | (1307) | | TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG | |
| gp 160 del 128-194 | (1352) | | TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG | |
| gp140TM | (1601) | | TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG | |
| gp140 | (1601) | | TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG | |
| gp140mut | (1601) | | TCATCGGCTTC-CTGGGCGCCGCCGGGAGCACCATGGGCG | |
| gp120 | (1583) | | ATATCGGATCCTCTAGA----- | |
| Consensus | (1601) | | TCATCGGCTTCNCTGGGCGCCGCCGGGAGCACCATGGGCG | |
| | | 1641 | | 1680 |
| gp160 | (1640) | | CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT | |
| gp160 del V1 | (1544) | | CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT | |
| gp160 del V2 | (1559) | | CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT | |
| gp160 del V1-2 | (1346) | | CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT | |
| gp 160 del 128-194 | (1391) | | CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT | |
| gp140TM | (1640) | | CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT | |
| gp140 | (1640) | | CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT | |
| gp140mut | (1640) | | CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT | |
| gp120 | (1600) | | ----- | |
| Consensus | (1641) | | CCGCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCT | |
| | | 1681 | | 1720 |
| gp160 | (1680) | | GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC | |
| gp160 del V1 | (1584) | | GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC | |
| gp160 del V2 | (1599) | | GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC | |
| gp160 del V1-2 | (1386) | | GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC | |
| gp 160 del 128-194 | (1431) | | GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC | |
| gp140TM | (1680) | | GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC | |
| gp140 | (1680) | | GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC | |
| gp140mut | (1680) | | GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC | |
| gp120 | (1600) | | ----- | |
| Consensus | (1681) | | GAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCC | |
| | | 1721 | | 1760 |
| gp160 | (1720) | | ATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG | |
| gp160 del V1 | (1624) | | ATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG | |
| gp160 del V2 | (1639) | | ATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG | |
| gp160 del V1-2 | (1426) | | ATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG | |
| gp 160 del 128-194 | (1471) | | ATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG | |
| gp140TM | (1720) | | ATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG | |
| gp140 | (1720) | | ATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG | |
| gp140mut | (1720) | | ATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG | |
| gp120 | (1600) | | ----- | |
| Consensus | (1721) | | ATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGG | |

FIG. 66B-9

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| | | | |
|--------------------|--------|---|------|
| | | 1761 | 1800 |
| gp160 | (1760) | GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG | |
| gp160 del V1 | (1664) | GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG | |
| gp160 del V2 | (1679) | GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG | |
| gp160 del V1-2 | (1466) | GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG | |
| gp 160 del 128-194 | (1511) | GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG | |
| gp140TM | (1760) | GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG | |
| gp140 | (1760) | GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG | |
| gp140mut | (1760) | GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG | |
| gp120 | (1600) | ----- | |
| Consensus | (1761) | GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG | 1840 |
| | | 1801 | |
| gp160 | (1800) | CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC | |
| gp160 del V1 | (1704) | CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC | |
| gp160 del V2 | (1719) | CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC | |
| gp160 del V1-2 | (1506) | CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC | |
| gp 160 del 128-194 | (1551) | CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC | |
| gp140TM | (1800) | CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC | |
| gp140 | (1800) | CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC | |
| gp140mut | (1800) | CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC | |
| gp120 | (1600) | ----- | |
| Consensus | (1801) | CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC | 1880 |
| | | 1841 | |
| gp160 | (1840) | AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA | |
| gp160 del V1 | (1744) | AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA | |
| gp160 del V2 | (1759) | AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA | |
| gp160 del V1-2 | (1546) | AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA | |
| gp 160 del 128-194 | (1591) | AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA | |
| gp140TM | (1840) | AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA | |
| gp140 | (1840) | AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA | |
| gp140mut | (1840) | AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA | |
| gp120 | (1600) | ----- | |
| Consensus | (1841) | AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA | 1920 |
| | | 1881 | |
| gp160 | (1880) | GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA | |
| gp160 del V1 | (1784) | GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA | |
| gp160 del V2 | (1799) | GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA | |
| gp160 del V1-2 | (1586) | GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA | |
| gp 160 del 128-194 | (1631) | GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA | |
| gp140TM | (1880) | GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA | |
| gp140 | (1880) | GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA | |
| gp140mut | (1880) | GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA | |
| gp120 | (1600) | ----- | |
| Consensus | (1881) | GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA | 1960 |
| | | 1921 | |
| gp160 | (1920) | CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC | |
| gp160 del V1 | (1824) | CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC | |
| gp160 del V2 | (1839) | CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC | |
| gp160 del V1-2 | (1626) | CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC | |
| gp 160 del 128-194 | (1671) | CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC | |
| gp140TM | (1920) | CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC | |
| gp140 | (1920) | CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC | |
| gp140mut | (1920) | CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC | |
| gp120 | (1600) | ----- | |
| Consensus | (1921) | CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC | |

FIG. 66B-10

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| | | | |
|--------------------|--------|--|------|
| | | | 2000 |
| | | 1961 | |
| gp160 | (1960) | ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC | |
| gp160 del V1 | (1864) | ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC | |
| gp160 del V2 | (1879) | ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC | |
| gp160 del V1-2 | (1666) | ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC | |
| gp 160 del 128-194 | (1711) | ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC | |
| gp140TM | (1960) | ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC | |
| gp140 | (1960) | ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC | |
| gp140mut | (1960) | ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC | |
| gp120 | (1600) | ----- | |
| Consensus | (1961) | ACCGGCCTGATCTACAACCTGATCGAGATCGCCCAGAACC | |
| | | 2001 | |
| gp160 | (2000) | AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA | |
| gp160 del V1 | (1904) | AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA | |
| gp160 del V2 | (1919) | AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA | |
| gp160 del V1-2 | (1706) | AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA | |
| gp 160 del 128-194 | (1751) | AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA | |
| gp140TM | (2000) | AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA | |
| gp140 | (2000) | AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA | |
| gp140mut | (2000) | AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA | |
| gp120 | (1600) | ----- | |
| Consensus | (2001) | AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAA | |
| | | 2041 | |
| gp160 | (2040) | GTGGGCCAGCCTGTGGAACCTGGTTTCGACATCACCAACTGG | |
| gp160 del V1 | (1944) | GTGGGCCAGCCTGTGGAACCTGGTTTCGACATCACCAACTGG | |
| gp160 del V2 | (1959) | GTGGGCCAGCCTGTGGAACCTGGTTTCGACATCACCAACTGG | |
| gp160 del V1-2 | (1746) | GTGGGCCAGCCTGTGGAACCTGGTTTCGACATCACCAACTGG | |
| gp 160 del 128-194 | (1791) | GTGGGCCAGCCTGTGGAACCTGGTTTCGACATCACCAACTGG | |
| gp140TM | (2040) | GTGGGCCAGCCTGTGGAACCTGGTTTCGACATCACCAACTGG | |
| gp140 | (2040) | GTGGGCCAGCCTGTGGAACCTGGTTTCGACATCACCAACTGG | |
| gp140mut | (2040) | GTGGGCCAGCCTGTGGAACCTGGTTTCGACATCACCAACTGG | |
| gp120 | (1600) | ----- | |
| Consensus | (2041) | GTGGGCCAGCCTGTGGAACCTGGTTTCGACATCACCAACTGG | |
| | | 2120 | |
| gp160 | (2080) | CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC | |
| gp160 del V1 | (1984) | CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC | |
| gp160 del V2 | (1999) | CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC | |
| gp160 del V1-2 | (1786) | CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC | |
| gp 160 del 128-194 | (1831) | CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC | |
| gp140TM | (2080) | CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC | |
| gp140 | (2080) | CTGTGGTACATC----- | |
| gp140mut | (2080) | CTGTGGTACATC----- | |
| gp120 | (1600) | ----- | |
| Consensus | (2081) | CTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGGCC | |
| | | 2121 | |
| gp160 | (2120) | TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCA---- | |
| gp160 del V1 | (2024) | TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCA---- | |
| gp160 del V2 | (2039) | TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCA---- | |
| gp160 del V1-2 | (1826) | TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCA---- | |
| gp 160 del 128-194 | (1871) | TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCA---- | |
| gp140TM | (2120) | TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCATCGT | |
| gp140 | (2092) | ----- | |
| gp140mut | (2092) | ----- | |
| gp120 | (1600) | ----- | |
| Consensus | (2121) | TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCANNNN | |

FIG. 66B-11

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| | | | |
|--------------------|--------|---|------|
| | | 2161 | 2200 |
| gp160 | (2156) | -TCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC | |
| gp160 del V1 | (2060) | -TCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC | |
| gp160 del V2 | (2075) | -TCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC | |
| gp160 del V1-2 | (1862) | -TCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC | |
| gp 160 del 128-194 | (1907) | -TCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC | |
| gp140TM | (2160) | GTAAGATATCGGATCCTCTAGA----- | |
| gp140 | (2092) | -TAAGATATCGGATCCTCTAGA----- | |
| gp140mut | (2092) | -TAAGATATCGGATCCTCTAGA----- | |
| gp120 | (1600) | ----- | |
| Consensus | (2161) | NTCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCC | |
| | | 2201 | 2240 |
| gp160 | (2195) | TGCAGACCCGCGCTGCCCCGCCAGCGCGGCCCCGACCGCCC | |
| gp160 del V1 | (2099) | TGCAGACCCGCGCTGCCCCGCCAGCGCGGCCCCGACCGCCC | |
| gp160 del V2 | (2114) | TGCAGACCCGCGCTGCCCCGCCAGCGCGGCCCCGACCGCCC | |
| gp160 del V1-2 | (1901) | TGCAGACCCGCGCTGCCCCGCCAGCGCGGCCCCGACCGCCC | |
| gp 160 del 128-194 | (1946) | TGCAGACCCGCGCTGCCCCGCCAGCGCGGCCCCGACCGCCC | |
| gp140TM | (2182) | ----- | |
| gp140 | (2113) | ----- | |
| gp140mut | (2113) | ----- | |
| gp120 | (1600) | ----- | |
| Consensus | (2201) | TGCAGACCCGCGCTGCCCCGCCAGCGCGGCCCCGACCGCCC | |
| | | 2241 | 2280 |
| gp160 | (2235) | CGAGGGCATCGAGGAGGAGGGCGGCAGCGCGACCGCGAC | |
| gp160 del V1 | (2139) | CGAGGGCATCGAGGAGGAGGGCGGCAGCGCGACCGCGAC | |
| gp160 del V2 | (2154) | CGAGGGCATCGAGGAGGAGGGCGGCAGCGCGACCGCGAC | |
| gp160 del V1-2 | (1941) | CGAGGGCATCGAGGAGGAGGGCGGCAGCGCGACCGCGAC | |
| gp 160 del 128-194 | (1986) | CGAGGGCATCGAGGAGGAGGGCGGCAGCGCGACCGCGAC | |
| gp140TM | (2182) | ----- | |
| gp140 | (2113) | ----- | |
| gp140mut | (2113) | ----- | |
| gp120 | (1600) | ----- | |
| Consensus | (2241) | CGAGGGCATCGAGGAGGAGGGCGGCAGCGCGACCGCGAC | |
| | | 2281 | 2320 |
| gp160 | (2275) | CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT | |
| gp160 del V1 | (2179) | CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT | |
| gp160 del V2 | (2194) | CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT | |
| gp160 del V1-2 | (1981) | CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT | |
| gp 160 del 128-194 | (2026) | CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT | |
| gp140TM | (2182) | ----- | |
| gp140 | (2113) | ----- | |
| gp140mut | (2113) | ----- | |
| gp120 | (1600) | ----- | |
| Consensus | (2281) | CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT | |
| | | 2321 | 2360 |
| gp160 | (2315) | GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG | |
| gp160 del V1 | (2219) | GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG | |
| gp160 del V2 | (2234) | GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG | |
| gp160 del V1-2 | (2021) | GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG | |
| gp 160 del 128-194 | (2066) | GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG | |
| gp140TM | (2182) | ----- | |
| gp140 | (2113) | ----- | |
| gp140mut | (2113) | ----- | |
| gp120 | (1600) | ----- | |
| Consensus | (2321) | GGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCG | |

FIG. 66B-12

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2361

2400

gp160 (2355) CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG

gp160 del V1 (2259) CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG

gp160 del V2 (2274) CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG

gp160 del V1-2 (2061) CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG

gp 160 del 128-194 (2106) CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG

gp140TM (2182) -----

gp140 (2113) -----

gp140mut (2113) -----

gp120 (1600) -----

Consensus (2361) CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG

2440

2401

gp160 (2395) CTGCTGGGCGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGT

gp160 del V1 (2299) CTGCTGGGCGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGT

gp160 del V2 (2314) CTGCTGGGCGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGT

gp160 del V1-2 (2101) CTGCTGGGCGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGT

gp 160 del 128-194 (2146) CTGCTGGGCGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGT

gp140TM (2182) -----

gp140 (2113) -----

gp140mut (2113) -----

gp120 (1600) -----

Consensus (2401) CTGCTGGGCGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGT

2480

2441

gp160 (2435) GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG

gp160 del V1 (2339) GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG

gp160 del V2 (2354) GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG

gp160 del V1-2 (2141) GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG

gp 160 del 128-194 (2186) GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG

gp140TM (2182) -----

gp140 (2113) -----

gp140mut (2113) -----

gp120 (1600) -----

Consensus (2441) GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG

2520

2481

gp160 (2475) CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC

gp160 del V1 (2379) CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC

gp160 del V2 (2394) CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC

gp160 del V1-2 (2181) CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC

gp 160 del 128-194 (2226) CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC

gp140TM (2182) -----

gp140 (2113) -----

gp140mut (2113) -----

gp120 (1600) -----

Consensus (2481) CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC

2560

2521

gp160 (2515) GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT

gp160 del V1 (2419) GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT

gp160 del V2 (2434) GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT

gp160 del V1-2 (2221) GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT

gp 160 del 128-194 (2266) GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT

gp140TM (2182) -----

gp140 (2113) -----

gp140mut (2113) -----

gp120 (1600) -----

Consensus (2521) GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT

FIG. 66B-13

SUBSTITUTE SHEET (RULE 26)

[illegible]

FIG. 66B-14

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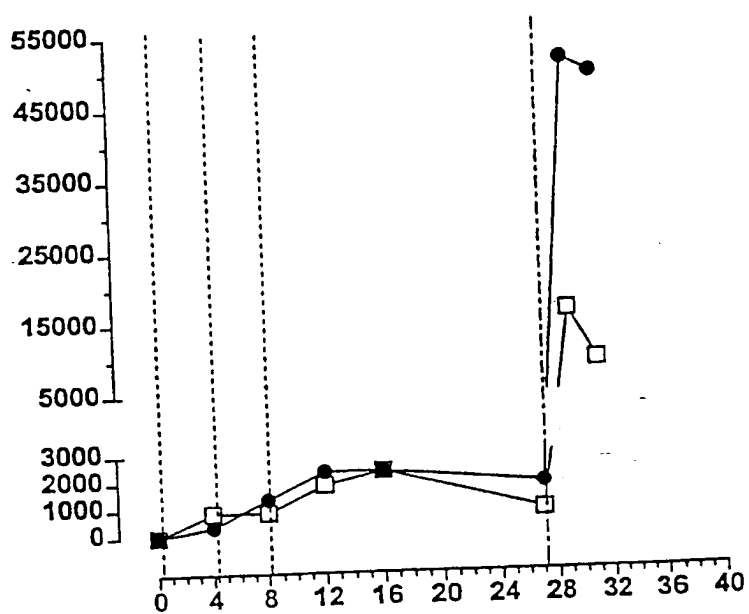


FIG. 67

SUBSTITUTE SHEET (RULE 26)

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HIV-1SF2 wt RT (PISPIET-->GIRKVL)

CCCATTAGTCCTATTGAACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAA
GTTAAGCAATGGCCATTGACAGAAGAAAAATAAAAGCATTAGTAGAGATATGTACAGAA
ATGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAGTA
TTTGCTATAAAGAAAAAGACAGTACTAAATGGAGAAAAGTAGTAGATTTTCAGAGAACTT
AATAAAAGAACTCAAGACTTCTGGGAAGTTCAGTTAGGAATACCACACCCCGCAGGGTTA
AAAAAGAAAAAATCAGTAACAGTATTGGATGTGGGTGATGCATACTTTTCAGTTCCCTTA
GATAAAGACTTTAGAAAGTATACTGCATTTACCATACTAGTATAAACAATGAGACACCA
GGGATTAGATATCAGTACAATGTGCTGCCACAGGGATGGAAAGGATCACCAGCAATATTC
CAAAGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAAACAGAATCCAGACATAGTTATC
TATCAAtacatggatgatTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACA
AAAATAGAGGAAGTGAACAGCATCTGTTGAGGTGGGGATTACCACACCAGACAAAAAA
CATCAGAAAGAACCTCCATTCCTTtggatgggttatGAACTCCATCCTGATAAATGGACA
GTACAGCCTATAATGCTGCCAGAAAAAGACAGCTGGACTGTCAATGACATACAGAAGTTA
GTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGATTAAAGTAAAGCAGTTATGT
AAACTCCTTAGAGGAACCAAAGCACTAACAGAAGTAATACCACTAACAGAAGAAGCAGAG
CTAGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCAGTACATGAAGTATATTATGAC
CCATCAAAAGACTTAGTAGCAGAAATACAGAAGCAGGGGCAAGGQCAATGGACATATCAA
ATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAGTATGCAAGGATGAGGGGTGCC
CACACTAATGATGTAAACAGTTAACAGAGGCAGTGCAAAAAGTATCCACAGAAAGCATA
GTAATATGGGGAAAGATTCCTAAATTTAAACTACCCATACAAAAGGAAACATGGGAAGCA
TGGTGGATGGAGTATTGGCAAGCTACCTGGATTCCCTGAGTGGGAGTTTGTCAATACCCCT
CCCTTAGTGAAATTATGGTACCAGTTAGAGAAAGAACCCATAGTAGGAGCAGAACTTTC
TATGTAGATGGGGCAGCTAATAGGGAGACTAAATTAGGAAAAGCAGGATATGTTACTGAC
AGAGGAAGACAAAAAGTTGTCTCCATAGCTGACACAACAAATCAGAAGACTGAATTACAA
GCAATTCATCTAGCTTTGCAGGATTGGGGATTAGAAGTAAACATAGTAACAGACTCACAA
TATGCATTAGGAATCATTCAAGCACAAACCAGATAAGAGTGAATCAGAGTTAGTCAGTCAA
ATAATAGAGCAGTTAATAAAAAAGGAAAAGGTCTACCTGGCATGGGTACCAGCACACAAA
GGAATTGGAGGAAATGAACAAGTAGATAAATTAGTCAGTGCTGGAATCAGGAAAGTACTA

FIG. 68

(SEQ ID NO:77)

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GagProtMod.SF2 (GP1)

[illegible]

FIG. 69
(SEO ID NO:78)

SUBSTITUTE SHEET (RULE 26)

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GagProtMod.SF2 (GP2)

GTCGACGCCACCATGGGCGCCCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGTGG
GAGAAGATCCGCCTGCGCCCCGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGG
GCCAGCCGCGAGCTGGAGCGCTTCGCCGTGAACCCCGGCCTGCTGGAGACCAGCGAGGGC
TGCCGCCAGATCCTGGGCCAGCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGC
AGCCTGTACAACACCGTGGCCACCCTGTACTGCGTGCACCAGCGCATCGACGTCAAGGAC
ACCAAGGAGGCCCTGGAGAAGATCGAGGAGGAGCAGAACAAGTCCAAGAAGAAGGCCAG
CAGGCCGCGCCGCCCGCCGGCACC GGCAACAGCAGCCAGGTGAGCCAGAACTACCCCATC
GTGCAGAACCTGCAGGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCCCTGAACGCC
TGGGTGAAGGTGGTGGAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCC
CTGAGCGAGGGCGCCACCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCAC
CAGGCCGCCATGCAGATGCTGAAGGAGACCATCAACGAGGAGGCCGCGAGTGGGACCGC
GTGCACCCCGTGCACGCCGGCCCCATCGCCCCCGGCCAGATGCGCGAGCCCCCGCGGCAGC
GACATCGCCGGCACCACCAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCAACAACCC
CCCATCCCCGTGGGCGAGATCTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCGTG
CGGATGTACAGCCCCACCAGCATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCCGC
GACTACGTGGACCGCTTCTACAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGAAG
AACTGGATGACCGAGACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTG
AAGGCTCTCGGCCCCGCGGCCACCCTGGAGGAGATGATGACCGCTGCCAGGGCGTGGGC
GGCCCCGGCCACAAGGCCCGCGTGCTGGCCGAGGCGATGAGGCAGGTGACGAACCCGGCG
ACCATCATGATGCAGCGCGGCAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAAC
TGCGGCAAGGAGGGCCACACCGCCAGGAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGG
CGCTGCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTA
GGGAAGATCTGGCCTTCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAG
CCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAGGAGAAAACAACTCCCTCTCAG
AAGCAGGAGCCGATAGACAAGGAACTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGC
AACGACCCCTCGTCAAGTAAGGATCGGGGGGCAACTCAAGGAAGCGCTGCTCGATACAG
GAGCAGATGATACAGTATTAGAAGAAATGAATTTGCCAGGAAAATGGAAACCAAAAATGA
TAGGGGGGATCGGGGGCTTCATCAAGGTGAGGCAGTACGACCAGATACCTGTAGAAATCT
GTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAA
GAAATCTGTTGACCCAGATCGGCTGCACCTTGAACCTCCCCATCAGCCCTATTGAGACGG
TGCCCGTGAAGTTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAATGGCCATTGTAAG
AATTC

FIG. 70

(SEQ ID NO:79)

SUBSTITUTE SHEET (RULE 26)

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FS(+)_ProtInact_RTopt_YM

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTAGGGA
AGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCAA
CAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAGGAGAAAACAACTCCCTCTCAGAAGC
AGGAGCCGATAGACAAGGAACTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGCAACG
ACCCCTCGTCAACAATAAGGATCGGGGGGCAACTCAAGGAAGCGCTGCTCGATACAGGAGC
AGATGATACAGTATTAGAAGAAATGAATTTGCCAGGAAAATGGAACCAAAAATGATAGG
GGGGATCGGGGGCTTCATCAAGGTGAGGCAGTACGACCAGATACCTGTAGAAATCTGTGG
ACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAA
TCTGTTGACCCAGATCGGCTGCACCTTGAACCTCCCCATCAGCCCTATTGAGACGGTGCC
CGTGAAGTTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAATGGCCATTGACCGAGGA
GAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCAA
GATCGGCCCCGAGAACCCTACAACACCCCCGTGTTCCGCATCAAGAAGAAGGACAGCAC
CAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGA
GGTGCAGCTGGGCATCCCCACCCCCGCCGCTGAAGAAGAAGAAGAGCGTGACCGTGCT
GGACGTGGGCGACGCCTACTTCAGCGTGCCCCCTGGACAAGGACTTCCGCAAGTACACCGC
CTTCACCATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAGTACAACGTGCT
GCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGGA
GCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGGCCCCCTGTACGTGGGCAG
CGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACCTGCTGCG
CTGGGGCTTCACCACCCCCGACAAGAAGCACCAGAAGGAGCCCCCTTCTGTGGATGGG
CTACGAGCTGCACCCCCGACAAGTGGACCGTGCAGCCCATCATGCTGCCCCGAGAAGGACAG
CTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCTA
CGCCGGCATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCGA
GGTGATCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGAA
GGAGCCCGTGCACGAGGTGTACTACGACCCCAGCAAGGACCTGGTGGCCGAGATCCAGAA
GCAGGGCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGAC
CGGCAAGTACGCCCGCATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGACCGAGGC
CGTGCAGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGTTCAAGCT

FIG. 71A

(SEQ ID NO:80)

SUBSTITUTE SHEET (RULE 26)

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GCCCATCCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCACCTGGAT
CCCCGAGTGGGAGTTCGTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAA
GGAGCCCATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACC GCGAGACCAA
GCTGGGCAAGGCCGGCTACGTGACCGACCGGGGCGGCAGAAAGGTGGTGAGCATCGCCGA
CACCACCAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACAGCGGCCT
GGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCCAGCCCGA
CAAGAGCGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGT
GTACCTGGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGGACAAGCT
GGTGAGCGCCGGCATCCGCAAGGTGCTGTTCTGAACGGCATCGATGGCGGCATCGTGAT
CTACCAGTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATTAAAGCT
TCCCGGGGCTAGCACCGGTGAATTC

FIG. 71B
(SEQ ID NO:80)

SUBSTITUTE SHEET (RULE 26)

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FS(+)_ProtInact_RTopt_YMWM

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTAGGGA
AGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCAA
CAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAGGAGAAAACAACTCCCTCTCAGAAGC
AGGAGCCGATAGACAAGGAAGTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGCAACG
ACCCCTCGTCACAATAAGGATCGGGGGGCAACTCAAGGAAGCGCTGCTCGATACAGGAGC
AGATGATACAGTATTAGAAGAAATGAATTTGCCAGGAAAATGGAAACCAAAATGATAGG
GGGGATCGGGGGCTTCATCAAGGTGAGGCAGTACGACCAGATACCTGTAGAAATCTGTGG
ACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAA
TCTGTTGACCCAGATCGGCTGCACCTTGAAGTTCCCATCAGCCCTATTGAGACGGTGCC
CGTGAAGTTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAATGGCCATTGACCGAGGA
GAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCAA
GATCGGGCCCCGAGAACCCCTACAACACCCCCGTGTTCCGATCAAGAAGAAGGACAGCAC
CAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGA
GGTGCAGCTGGGCATCCCCACCCCGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGCT
GGACGTGGGCGACGCCTACTTCAGCGTGCCCTGGACAAGGACTTCCGCAAGTACACCGC
CTTCACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAGTACAACGTGCT
GCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGGA
GCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGGCCCCCCTGTACGTGGGCAG
CGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACCTGCTGCG
CTGGGGCTTACCACCCCCGACAAGAAGCACCAGAAGGAGCCCCCCTTCCCTGCCCATCGA
GCTGCACCCCCGACAAGTGGACCGTGCAGCCCATCATGCTGCCCCGAGAAGGACAGCTGGAC
CGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCTACGCCGG
CATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCGAGGTGAT
CCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGAAGGAGCC
CGTGCACGAGGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGG
CCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCAA
GTACGCCCGCATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCA
GAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGTTCAAGCTGCCCAT

FIG. 72A

(SEQ ID NO:81)

SUBSTITUTE SHEET (RULE 26)

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CCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCACCTGGATCCCCGA
GTGGGAGTTTCGTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCC
CATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACC GCGAGACCAAGCTGGG
CAAGGCCGGCTACGTGACCGACCGGGGCCGGCAGAAGGTGGTGAGCATCGCCGACACCAC
CAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACAGCGGCCTGGAGGT
GAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCCAGCCCGACAAGAG
CGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCT
GGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGGACAAGCTGGTGAG
CGCCGGCATCCGCAAGGTGCTGTTCCCTGAACGGCATCGATGGCGGCATCGTGATCTACCA
GTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATTAAAAGCTTCCCGG
GGCTAGCACCGGTGAATTC

FIG. 72B

(SEQ ID NO:81)

SUBSTITUTE SHEET (RULE 26)

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FS(-)_ProtMod_RTpt_YM

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTCTTCCGCC
AGGACCTGGCCTTCCTGCAGGGCAAGGCCCCGCGAGTTCAGCAGCGAGCAGACCCGCGCCA
ACAGCCCCACCCGCCGCGAGCTGCAGGTGTGGGGCGGCGAGAACAACAGCCTGAGCGAGG
CCGGCGCCGACCGCCAGGGCACCCTGAGCTTCAACTTCCCCCAGATCACCTGTGGCAGC
GCCCCCTGGTGACCATCAGGATCGGCGGCCAGCTCAAGGAGGCGCTGCTCGACACCGGCG
CCGACGACACCGTGCTGGAGGAGATGAACCTGCCCCGCAAGTGGAAGCCCAAGATGATCG
GCGGGATCGGGGGCTTCATCAAGGTGCGGCAGTACGACCAGATCCCCGTGGAGATCTGCG
GCCACAAGGCCATCGGCACCGTGCTGGTGGGCCCCACCCCCGTGAACATCATCGGCCGCA
ACCTGCTGACCCAGATCGGCTGCACCCTGAACTTCCCCATCAGCCCCATCGAGACGGTG
CCGTGAAGCTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCCCTGACCGAGG
AGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCA
AGATCGGCCCCGAGAACCCTACAACACCCCCGTGTTCCGCATCAAGAAGAAGGACAGCA
CCAAGTGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGG
AGGTGCAGCTGGGCATCCCCACCCCGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGC
TGGACGTGGGCGACGCCTACTTCAGCGTGCCCCCTGGACAAGGACTTCCGCAAGTACACCG
CCTTCACCATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAGTACAACGTGC
TGCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGG
AGCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGGCCCCCCTGTACGTGGGCA
GCGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACCTGCTGC
GCTGGGGCTTCACCACCCCCGACAAGAAGCACCAGAAGGAGCCCCCTTCCTGTGGATGG
GCTACGAGCTGCACCCCGACAAGTGGACCGTGACGCCATCATGCTGCCCCGAGAAGGACA
GCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCT
ACGCCGGCATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCG
AGGTGATCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGA
AGGAGCCCGTGACGAGGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGA
AGCAGGGCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGA
CCGGCAAGTACGCCCCGATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGACCGAGG
CCGTGCAGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGTTCAAGC

FIG. 73A

(SEQ ID NO:82)

SUBSTITUTE SHEET (RULE 26)

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TGCCCCATCCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCACCTGGA
TCCCCGAGTGGGAGTTCGTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGA
AGGAGCCCCATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACC GCGAGACCA
AGCTGGGCAAGGCCGGCTACGTGACCGACCGGGGCCGAGAGGTGGTGAGCATCGCCG
ACACCACCAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACAGCGGCC
TGGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCAGCCCG
ACAAGAGCGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGG
TGTACCTGGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGGACAAGC
TGGTGAGCGCCGGCATCCGCAAGGTGCTGTTTCCTGAACGGCATCGATGGCGGCATCGTGA
TCTACCAGTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATTAAAGC
TTCCCCGGGGCTAGCACCGGTGAATTC

FIG. 73B

(SEQ ID NO:82)

SUBSTITUTE SHEET (RULE 26)

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FS(-)_ProtMod_RTpt_YMWM

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTCTTCCGCG
AGGACCTGGCCTTCTCTGCAGGGCAAGGCCCGCGAGTTCAGCAGCGAGCAGACCCGCGCCA
ACAGCCCCACCCGCGCGAGCTGCAGGTGTGGGGCGGCGAGAACAACAGCCTGAGCGAGG
CCGGCGCCGACCGCCAGGGCACCGTGAGCTTCAACTTCCCCCAGATCACCTGTGGCAGC
GCCCCCTGGTGACCATCAGGATCGGCGGCCAGCTCAAGGAGGCGCTGCTCGACACCGGCG
CCGACGACACCGTGCTGGAGGAGATGAACCTGCCCCGCAAGTGGAAGCCCAAGATGATCG
GCGGGATCGGGGGCTTCATCAAGGTGCGGCAGTACGACCAGATCCCCGTGGAGATCTGCG
GCCACAAGGCCATCGGCACCGTGCTGGTGGGGCCCCACCCCGTGAACATCATCGGCCGCA
ACCTGCTGACCCAGATCGGCTGCACCCTGAACTTCCCCATCAGCCCCATCGAGACGGTGC
CCGTGAAGCTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCCCTGACCGAGG
AGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCA
AGATCGGCCCCGAGAACCCTTACAACACCCCGTGTTTCGCCATCAAGAAGAAGGACAGCA
CCAAGTGGCGGAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGG
AGGTGCAGCTGGGCATCCCCCACCCCGCGGCCTGAAGAAGAAGAAGAGCGTGACCGTG
TGGACGTGGGCGACGCCTACTTCAGCGTGCCCCTGGACAAGGACTTCCGCAAGTACACCG
CCTTCACCATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAGTACAACGTGC
TGCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGG
AGCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGGCCCCCCTGTACGTGGGCA
GCGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACCTGCTGC
GCTGGGGCTTCAACACCCCGACAAGAAGCACCAAGGAGCCCCCCTTCTGCCCATCG
AGCTGCACCCCGACAAGTGGACCGTGAGCCCATCATGCTGCCCCGAGAAGGACAGCTGGA
CCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCTACGCCG
GCATCAAGGTGAAGCAGCTGTGCAAGCTGTGCGCGGCACCAAGGCCCTGACCGAGGTGA
TCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGAAGGAGC
CCGTGCACGAGGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGG
GCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCA
AGTACGCCCCGATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGC
AGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGTTCAAGCTGCCCA

FIG. 74A

(SEQ ID NO:83)

SUBSTITUTE SHEET (RULE 26)

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TCCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCACCTGGATCCCCG
AGTGGGAGTTCGTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGC
CCATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACC GCGAGACCAAGCTGG
GCAAGGCCGGCTACGTGACCGACCGGGGCCGGCAGAAGGTGGTGAGCATCGCCGACACCA
CCAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACAGCGGCCTGGAGG
TGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCAGCCCGACAAGA
GCGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACC
TGGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGGACAAGCTGGTGA
GCGCCGGCATCCGCAAGGTGCTGTTCTGAACGGCATCGATGGCGGCATCGTGATCTACC
AGTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATTAAAAGCTTCCCCG
GGGCTAGCACCGGTGAATTC

FIG. 74B

(SEQ ID NO:83)

SUBSTITUTE SHEET (RULE 26)

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FS(-)_ProtMod_RTpt(+)

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTCTTCCGCG
AGGACCTGGCCTTCCTGCAGGGCAAGGCCCGCGAGTTCAGCAGCGAGCAGACCCGCGCCA
ACAGCCCCACCCGCGCGAGCTGCAGGTGTGGGGCGGCGAGAACAACAGCCTGAGCGAGG
CCGGCGCCGACCGCCAGGGCACCGTGAGCTTCAACTTCCCCCAGATCACCTGTGGCAGC
GCCCCCTGGTGACCATCAGGATCGGCGGCCAGCTCAAGGAGGCGCTGCTCGACACCGGCG
CCGACGACACCGTGCTGGAGGAGATGAACCTGCCCGGCAAGTGGAAGCCCAAGATGATCG
GCGGGATCGGGGGCTTCATCAAGGTGCGGCAGTACGACCAGATCCCCGTGGAGATCTGCG
GCCACAAGGCCATCGGCACCGTGCTGGTGGGCCCCACCCCGTGAACATCATCGGCCGCA
ACCTGCTGACCCAGATCGGCTGCACCCTGAACTTCCCCATCAGCCCCATCGAGACGGTG
CCGTGAAGCTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCCCTGACCGAGG
AGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCA
AGATCGGCCCCGAGAACCCTACAACACCCCGTGTTGCCATCAAGAAGAAGGACAGCA
CCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGG
AGGTGCAGCTGGGCATCCCCACCCCGCGGCCCTGAAGAAGAAGAAGAGCGTGACCGTG
TGGACGTGGGCGACGCTACTTCAGCGTGCCCTGGACAAGGACTTCCGCAAGTACACCG
CCTTACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAGTACAACGTGC
TGCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCAGAGCAGCATGACCAAGATCCTGG
AGCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGTACATGGACGACCTGTACG
TGGGCAGCGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACC
TGCTGCGCTGGGGCTTACCACCCCGACAAGAAGCACCAGAAGGAGCCCCCTTCTGT
GGATGGGCTACGAGCTGCACCCCGACAAGTGACCGTGACGCCATCATGCTGCCCCGAGA
AGGACAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCC
AGATCTACCGCGCATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCC
TGACCGAGGTGATCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGA
TCCTGAAGGAGCCCGTGACGAGGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGA
TCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACC
TGAAGACCGGCAAGTACGCCCCGATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGA
CCGAGGCCGTGCAGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGT
TCAAGCTGCCCATCCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCA
CCTGGATCCCCGAGTGGGAGTTCGTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGC
TGGAGAAGGAGCCCATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACCGCG

FIG. 75A

(SEQ ID NO:84)

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AGACCAAGCTGGGCAAGGCCGGCTACGTGACCGACCGGGGCCGGCAGAAGGTGGTGAGCA
TCGCCGACACCACCAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACA
GCGGCCTGGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCC
AGCCCGACAAGAGCGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGG
AGAAGGTGTACCTGGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGG
ACAAGCTGGTGAGCGCCGGCATCCGCAAGGTGCTGTTCTGAACGGCATCGATGGCGGCA
TCGTGATCTACCAGTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATT
AAAAGCTTCCCGGGGCTAGCACCGGTGAATTC

FIG. 75B

(SEQ ID NO:84)

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Tat_wt_SF162 (wildtype)

ATGGAGCCAGTAGATCCTAGATTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAGA
CTGCTTGTAACAAATTGCTATTGTAAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTCATAAC
AAAAGGCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCCT
CCAGACAGTGAGGTTTCATCAAGTTTCTCTACCAAAGCAACCCGCTTCCCAGCCCCAAGG
GGACCCGACAGGCCCGAAGGAATCGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGA
TCCAGTCCATTAG

FIG. 76

(SEQ ID NO:85)

Tat_SF162

MEPVDPRLPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKGLGISYGRKKRRQRRRAPDSE
VHQVSLPKQPASQPQGDPTGPKESKKKVERETETDPVH

FIG. 77

(SEQ ID NO:86)

Tat_SF162_opt

ATGGAGCCCGTGGACCCCCGCCTGGAGCCCTGGAAGCACCCCGGCAGCCAGCCCAAGAC
CGCCTGCACCAACTGCTACTGCAAGAAGTGCTGCTTCCACTGCCAGGTGTGCTTCATCACC
AAGGGCCTGGGCATCAGCTACGGCCGCAAGAAGCGCCGCCAGCGCCGCCGCGCCCCCCCC
CGACAGCGAGGTGCACCAGGTGAGCCTGCCCAAGCAGCCCGCCAGCCAGCCCCAGGGCG
ACCCACCGGCCCAAGGAGAGCAAGAAGAAGGTGGAGCGCGAGACCGAGACCGACCCCG
GTGCACTAG

FIG. 78

(SEQ ID NO:87)

Tat_Cys22_SF162_opt

ATGGAGCCCGTGGACCCCCGCCTGGAGCCCTGGAAGCACCCCGGCAGCCAGCCCAAGAC
CGCCGACCAACTGCTACTGCAAGAAGTGCTGCTTCCACTGCCAGGTGTGCTTCATCACCA
AGGGCCTGGGCATCAGCTACGGCCGCAAGAAGCGCCGCCAGCGCCGCCGCGCCCCCCCC
GACAGCGAGGTGCACCAGGTGAGCCTGCCCAAGCAGCCCGCCAGCCAGCCCCAGGGCGA
CCCCACCGGCCCAAGGAGAGCAAGAAGAAGGTGGAGCGCGAGACCGAGACCGACCCCG
TGCCTAG

FIG. 79

(SEQ ID NO:88)

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Alignment GagMod vs GP1_GP2

| | | | | | | | | | | Section 6 |
|----------------------|-------|--|-----|-----|-----|-----|-----|-----|-----|------------|
| | | | | | | | | | | |
| | (381) | 381 | 390 | 400 | 410 | 420 | 430 | 440 | 456 | |
| GagMod.SF2 | (381) | CAGCCAGGTGAGCCAGAACTACCCCATCGTGAGAACTGAGGGCAGATGGTGCAACAGGCCATCAGCCCCCGC | | | | | | | | |
| GagProtMod.SF2 (GP1) | (381) | CAGCCAGGTGAGCCAGAACTACCCCATCGTGAGAACTGAGGGCAGATGGTGCAACAGGCCATCAGCCCCCGC | | | | | | | | |
| GagProtMod.SF2 (GP2) | (381) | CAGCCAGGTGAGCCAGAACTACCCCATCGTGAGAACTGAGGGCAGATGGTGCAACAGGCCATCAGCCCCCGC | | | | | | | | |
| Consensus | (381) | CAGCCAGGTGAGCCAGAACTACCCCATCGTGAGAACTGAGGGCAGATGGTGCAACAGGCCATCAGCCCCCGC | | | | | | | | |
| | | | | | | | | | | Section 7 |
| | (457) | 457 | 470 | 480 | 490 | 500 | 510 | 520 | 532 | |
| GagMod.SF2 | (457) | ACCCTGAACGCTGGGTGAGGTGGTGGAGGAGAGGGCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCCCTGA | | | | | | | | |
| GagProtMod.SF2 (GP1) | (457) | ACCCTGAACGCTGGGTGAGGTGGTGGAGGAGAGGGCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCCCTGA | | | | | | | | |
| GagProtMod.SF2 (GP2) | (457) | ACCCTGAACGCTGGGTGAGGTGGTGGAGGAGAGGGCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCCCTGA | | | | | | | | |
| Consensus | (457) | ACCCTGAACGCTGGGTGAGGTGGTGGAGGAGAGGGCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCCCTGA | | | | | | | | |
| | | | | | | | | | | Section 8 |
| | (533) | 533 | 540 | 550 | 560 | 570 | 580 | 590 | 608 | |
| GagMod.SF2 | (533) | GCGAGGGCGCCACCCCCCAGGACCTGAACACGATGTTGAACACCCGTGGCGGGCCACCCAGGCCGCCATGCAGATGCT | | | | | | | | |
| GagProtMod.SF2 (GP1) | (533) | GCGAGGGCGCCACCCCCCAGGACCTGAACACGATGTTGAACACCCGTGGCGGGCCACCCAGGCCGCCATGCAGATGCT | | | | | | | | |
| GagProtMod.SF2 (GP2) | (533) | GCGAGGGCGCCACCCCCCAGGACCTGAACACGATGTTGAACACCCGTGGCGGGCCACCCAGGCCGCCATGCAGATGCT | | | | | | | | |
| Consensus | (533) | GCGAGGGCGCCACCCCCCAGGACCTGAACACGATGTTGAACACCCGTGGCGGGCCACCCAGGCCGCCATGCAGATGCT | | | | | | | | |
| | | | | | | | | | | Section 9 |
| | (609) | 609 | 620 | 630 | 640 | 650 | 660 | 670 | 684 | |
| GagMod.SF2 | (609) | GAAGGAGACCATCAACGAGGAGGCGCCCGCGAGTGGACCCGTCACCCCGTGCACGGCGGCCCATCGCCCCCGGC | | | | | | | | |
| GagProtMod.SF2 (GP1) | (609) | GAAGGAGACCATCAACGAGGAGGCGCCCGCGAGTGGACCCGTCACCCCGTGCACGGCGGCCCATCGCCCCCGGC | | | | | | | | |
| GagProtMod.SF2 (GP2) | (609) | GAAGGAGACCATCAACGAGGAGGCGCCCGCGAGTGGACCCGTCACCCCGTGCACGGCGGCCCATCGCCCCCGGC | | | | | | | | |
| Consensus | (609) | GAAGGAGACCATCAACGAGGAGGCGCCCGCGAGTGGACCCGTCACCCCGTGCACGGCGGCCCATCGCCCCCGGC | | | | | | | | |
| | | | | | | | | | | Section 10 |
| | (685) | 685 | 690 | 700 | 710 | 720 | 730 | 740 | 750 | 760 |
| GagMod.SF2 | (685) | CAGATGCGGAGAGCCCGCGGCGAGCATCGCCGGCACCCAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCA | | | | | | | | |
| GagProtMod.SF2 (GP1) | (685) | CAGATGCGGAGAGCCCGCGGCGAGCATCGCCGGCACCCAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCA | | | | | | | | |
| GagProtMod.SF2 (GP2) | (685) | CAGATGCGGAGAGCCCGCGGCGAGCATCGCCGGCACCCAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCA | | | | | | | | |
| Consensus | (685) | CAGATGCGGAGAGCCCGCGGCGAGCATCGCCGGCACCCAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCA | | | | | | | | |

FIG. 80B

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Alignment GagMod vs GP1_GP2

| | (761) | 761 | 770 | 780 | 790 | 800 | 810 | 820 | Section 11 |
|----------------------|--------|------|------|------|------|------|------|------|------------|
| GagMod.SF2 | (761) | 761 | 770 | 780 | 790 | 800 | 810 | 820 | 836 |
| GagProtMod.SF2 (GP1) | (761) | 761 | 770 | 780 | 790 | 800 | 810 | 820 | 836 |
| GagProtMod.SF2 (GP2) | (761) | 761 | 770 | 780 | 790 | 800 | 810 | 820 | 836 |
| Consensus | (761) | 761 | 770 | 780 | 790 | 800 | 810 | 820 | 836 |
| | (837) | 837 | 850 | 860 | 870 | 880 | 890 | 900 | 912 |
| GagMod.SF2 | (837) | 837 | 850 | 860 | 870 | 880 | 890 | 900 | 912 |
| GagProtMod.SF2 (GP1) | (837) | 837 | 850 | 860 | 870 | 880 | 890 | 900 | 912 |
| GagProtMod.SF2 (GP2) | (837) | 837 | 850 | 860 | 870 | 880 | 890 | 900 | 912 |
| Consensus | (837) | 837 | 850 | 860 | 870 | 880 | 890 | 900 | 912 |
| | (913) | 913 | 920 | 930 | 940 | 950 | 960 | 970 | 988 |
| GagMod.SF2 | (913) | 913 | 920 | 930 | 940 | 950 | 960 | 970 | 988 |
| GagProtMod.SF2 (GP1) | (913) | 913 | 920 | 930 | 940 | 950 | 960 | 970 | 988 |
| GagProtMod.SF2 (GP2) | (913) | 913 | 920 | 930 | 940 | 950 | 960 | 970 | 988 |
| Consensus | (913) | 913 | 920 | 930 | 940 | 950 | 960 | 970 | 988 |
| | (989) | 989 | 1000 | 1010 | 1020 | 1030 | 1040 | 1050 | 1064 |
| GagMod.SF2 | (989) | 989 | 1000 | 1010 | 1020 | 1030 | 1040 | 1050 | 1064 |
| GagProtMod.SF2 (GP1) | (989) | 989 | 1000 | 1010 | 1020 | 1030 | 1040 | 1050 | 1064 |
| GagProtMod.SF2 (GP2) | (989) | 989 | 1000 | 1010 | 1020 | 1030 | 1040 | 1050 | 1064 |
| Consensus | (989) | 989 | 1000 | 1010 | 1020 | 1030 | 1040 | 1050 | 1064 |
| | (1065) | 1065 | 1070 | 1080 | 1090 | 1100 | 1110 | 1120 | 1130 |
| GagMod.SF2 | (1065) | 1065 | 1070 | 1080 | 1090 | 1100 | 1110 | 1120 | 1130 |
| GagProtMod.SF2 (GP1) | (1065) | 1065 | 1070 | 1080 | 1090 | 1100 | 1110 | 1120 | 1130 |
| GagProtMod.SF2 (GP2) | (1065) | 1065 | 1070 | 1080 | 1090 | 1100 | 1110 | 1120 | 1130 |
| Consensus | (1065) | 1065 | 1070 | 1080 | 1090 | 1100 | 1110 | 1120 | 1130 |

FIG. 80C

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Alignment GagMod vs GP1_GP2

| | 1521 | 1530 | 1540 | 1550 | 1560 | 1570 | 1580 | 1596 | Section 21 |
|---------------------------|--|-------|-------|-------|-------|-------|-------|-------|------------|
| GagMod.SF2(1510) | (1521) | 1521 | 1530 | 1540 | 1550 | 1560 | 1570 | 1580 | 1596 |
| GagProtMod.SF2(GP1)(1521) | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| GagProtMod.SF2(GP2)(1521) | CAGCTCAAGGAGCGCTGCTCGACACCGCGCGGCGGAGATGAACCTGCCCGGCAAGTGGA | | | | | | | | |
| GagProtMod.SF2(GP2)(1521) | CAACTCAAGGAAGCGCTGCTCGATACAGGACGATGATACAGTATTAGAGAAATGAATTTGCCAGGAAATGGA | | | | | | | | |
| Consensus(1521) | CA CTAAGGA GCGTCTCTGA AC GG GC GA GA AC GT T GA GA ATGAA TGCC GG AA TGGA | | | | | | | | |
| GagMod.SF2(1510) | (1597) | 1597 | 1610 | 1620 | 1630 | 1640 | 1650 | 1660 | 1672 |
| GagProtMod.SF2(GP1)(1597) | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| GagProtMod.SF2(GP2)(1597) | AGCCCAAGATGATCGGCGGGATCGGGGCTTCATCAAGTGGGCGAGTACGACCAGATCCCCGTGGAGATCTGGCG | | | | | | | | |
| Consensus(1597) | AACCAAAATGATAGGGGATCGGGGCTTCATCAAGTGGGCGAGTACGACCAGATCCCCGTGGAGATCTGGCG | | | | | | | | |
| GagMod.SF2(1510) | (1673) | 1673 | 1680 | 1690 | 1700 | 1710 | 1720 | 1730 | 1748 |
| GagProtMod.SF2(GP1)(1673) | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| GagProtMod.SF2(GP2)(1673) | CCACAAGGCCATCGGCGCCGTCGTGGTGGGCCACCCCGTGAACATCATCGCGCGCAACCTGCTGACCCAGATC | | | | | | | | |
| Consensus(1673) | ACATAAGCTATAGGTACAGTATTAGTAGGACCTACCTGTCAACATAATTGGAAGAAATCTGTGACCCAGATC | | | | | | | | |
| GagMod.SF2(1510) | (1749) | 1749 | 1760 | 1770 | 1780 | 1790 | 1800 | 1810 | 1824 |
| GagProtMod.SF2(GP1)(1749) | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| GagProtMod.SF2(GP2)(1749) | GGCTGCACCCCTGAACCTTCCCATCAGCCCCATCGAGACGGTCCCCGTGAAGCTGAAGCCGGGATGGACGGCCCCA | | | | | | | | |
| Consensus(1749) | GGCTGCACCCCTGAACCTTCCCATCAGCCCCATCGAGACGGTCCCCGTGAAGCTGAAGCCGGGATGGACGGCCCCA | | | | | | | | |
| GagMod.SF2(1510) | (1825) | 1825 | 1830 | 1847 | | | | | |
| GagProtMod.SF2(GP1)(1825) | ----- | ----- | ----- | ----- | | | | | |
| GagProtMod.SF2(GP2)(1825) | AGGTCAAGCAGTGGCCCTGTAA | | | | | | | | |
| Consensus(1825) | AGGTCAAGCAGTGGCCCTGTAA | | | | | | | | |

FIG. 80E

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TataminoSF162.opt

ATGGAGCCCCGTGGACCCCCCGCCCTGGAGCCCTGGAGCGCAACCCCGGCAGCCAGCCCCAA
GACCGCCTGGACCAACTGCTACTGCAAGAAAGTGCTGCTTCCACTGCCAGGTGTGCTT
CATCACCAAGGGCCTGGGCATCAGCTACGGCCGCAAGAACGGCCGCCAGCGCCGC

FIG. 81
(SEQ ID NO:89)

Tat_Cys22_SF162

MEPVDPRLEPWKHPSQPKTAGTNCYCKKCCFHCQVCFITKGLGISYGRKKRRRRRAPPDSE
VHQVSLPKQPASQPQDPTGPKESKKKVERETETDPVHZ

FIG. 82
(SEQ ID NO:90)

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SEQUENCE LISTING

<110> Chiron Corporation

<120> IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND PRODUCTION
OF VIRUS-LIKE PARTICLES

<130> 1621.100

<140>

<141>

<160> 90

<170> PatentIn Ver. 2.0

<210> 1

<211> 1509

<212> DNA

<213> Human immunodeficiency virus

<400> 1

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ctagaacgat tcgcagtcaa tcctggcctg ttagaaacat cagaaggctg cagacaaata 180
ttgggacagc tacagccatc ccttcagaca ggatcagaag aacttagatc attatataat 240
acagtagcaa cctctatttg tgtacatcaa aggatagatg taaaagacac caaggaagct 300
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cagggggcaaa tgggtacatca ggccatatca cctagaactt taaatgcatg ggtaaaagta 480
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<211> 1845

<212> DNA

<213> Human immunodeficiency virus

<400> 2

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ctagaacgat tcgcagtcaa tcctggcctg ttagaaacat cagaaggctg cagacaaata 180
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<210> 3

<211> 4313

<212> DNA

<213> Human immunodeficiency virus

<400> 3

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```

<210> 4

<211> 1515

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
HIV-Gag

<400> 4


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1515

<210> 5

<211> 1853

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
HIV-Gag-protease

<400> 5

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<210> 6

<211> 4319

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic

HIV-Gag-polymerase

<400> 6

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cgcgagctgg agcgtctcgc cgtgaacccc ggctgctgg agaccagcga gggctgccgc 180
cagatcctgg gccagctgca gccagcctg cagaccggca gcgaggagct gcgcagcctg 240
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<210> 7

<211> 2031

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
HIV-Gag/HCV-core fusion polypeptide

<400> 7

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```

<210> 8

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
HIV-Gag/HCV-Core fusion polypeptide

<400> 8

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ggtagacgtc agcctatccc caaggctcgt cggcccagg gcaggacctg ggctcagccc 1740

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ggtaaggtca tcgataccct tacgtgcggc ttccgagacc tcatggggtg cataccgctc 1920
gtcggcgccc ctcttggagg cgctgccagg gccctggcgc atggcgccg ggttctggaa 1980
gacggcgtga actatgcaac agggaaacct cctggttgct cttag 2025

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<210> 9

<211> 1268

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic Gag
common region

<400> 9

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cgcgagctgg agcgcttcgc cgtgaacccc ggctgctgga agaccagcga gggctgcccgc 180
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gaggccctgg agaagatcga ggaggagcag aacaagtcca agaagaaggc ccagcaggcc 360
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atgatgcagc gcggcaactt ccgcaaccag cggaaagacc tcaagtgctt caactgcggc 1200
aaggagggcc acaccgccag gaactgcccg gcccccgca agaagggctg ctggcgctgc 1260
ggccgcga 1268

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<210> 10

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: HIV-Gag
peptide p7G

<400> 10

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Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu
  1             5             10             15

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Glu Ala Ala Glu
      20

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<210> 11

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer GAG5

<400> 11

aagaattcca tgggtgag agcgtcggtta

30

<210> 12

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer
p55-SAL3

<400> 12

attcgtcgac tgtgacgagg ggtcgttgcc

30

<210> 13

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer
CORESAL5

<400> 13

atttgcgac gaatcctaaa cctcaaagaa aaac

34

<210> 14

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer 173CORE

<400> 14

tattggatcc taagagcaac caggaagggtt c

31

<210> 15

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer MS65

<400> 15

cgaccatcat ggatgcagcg c

21

<210> 16

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer MS66

<400> 16

aggattcgtc gagtcgctgc tggggtcgtt

30

<210> 17

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer XPANXNF

<400> 17

gcacgtgggc ccggcgcttc tagagc

26

<210> 18

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer XPANXNR

<400> 18

gctctagagg cgccgggccc acgtgc

26

<210> 19

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: HIV p55 Gag
Major Homology Region

<400> 19

Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg
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Phe Tyr Lys Thr

20

<210> 20

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic p55
Gag Major Homology Region

<400> 20

gacatccgcc agggccccaaggagcccttc cgcgactacg tggaccgctt ctacaagacc 60

<210> 21

<211> 15

<212> PRT
<213> Human immunodeficiency virus

<400> 21
Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg
1 5 10 15

<210> 22
<211> 5
<212> PRT
<213> Human immunodeficiency virus

<400> 22
Lys Ala Lys Arg Arg
1 5

<210> 23
<211> 4
<212> PRT
<213> Human immunodeficiency virus

<400> 23
Arg Glu Lys Arg
1

<210> 24
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: aa of
mut7.SF162 cleavage site

<400> 24
Ala Pro Thr Lys Ala Ile Ser Ser Val Val Gln Ser Glu Lys Ser
1 5 10 15

<210> 25
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: aa of
mut8.SF162 cleavage site

<400> 25
Ala Pro Thr Ile Ala Ile Ser Ser Val Val Gln Ser Glu Lys Ser
1 5 10 15

<210> 26
<211> 15
<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: aa of
mut.SF162 cleavage site

<400> 26

Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Ser
1 5 10 15

<210> 27

<211> 15

<212> PRT

<213> Human immunodeficiency virus

<220>

<223> Description of Artificial Sequence: aa of native
cleavage site in US4

<400> 27

Ala Pro Thr Gln Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg
1 5 10 15

<210> 28

<211> 5

<212> PRT

<213> Human immunodeficiency virus

<220>

<223> Description of Artificial Sequence: aa of second
cleavage site in US4

<400> 28

Gln Ala Lys Arg Arg
1 5

<210> 29

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: aa of mut.US4
cleavage site

<400> 29

Ala Pro Thr Gln Ala Lys Arg Arg Val Val Gln Arg Glu Lys Ser
1 5 10 15

<210> 30

<211> 1419

<212> DNA

<213> Human immunodeficiency virus

<400> 30

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acacatgcct gtgtaccacac agaccctaac ccacaagaaa tagtattgga aaatgtgaca 180
gaaaaatttta acatgtggaa aaataacatg gtagaacaga tgcattgagga tataatcagt 240
ttatggggtac aaagtctaaa gccatgtgta aagttaaccc cactctgtgt tactctacat 300
tgcactaatt tgaagaatgc tactaataacc aagagtagta attggaaaga gatggacaga 360
ggagaaataa aaaattgctc tttcaaggtc accacaagca taagaaataa gatgcagaaa 420
gaatatgcac ttttttataa acttgatgta gtaccaatag ataattgataa tacaagctat 480
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agaggacaaa ttagatgctc atcaaatatt acaggactgc tattaacaag agatgggtgt 1260
aaagagatca gtaacaccac cgagatcttc agacctggag gtggagatat gagggacaa 1320
tggagaagtg aattatataa atataaagta gtaaaaattg agccattagg agtagcacc 1380
accaaggcaa agagaagagt ggtgcagaga gaaaaaaga 1419

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<210> 31

<211> 1932

<212> DNA

<213> Human immunodeficiency virus

<400> 31

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acacatgcct gtgtaccacac agaccctaac ccacaagaaa tagtattgga aaatgtgaca 180
gaaaaatttta acatgtggaa aaataacatg gtagaacaga tgcattgagga tataatcagt 240
ttatggggtac aaagtctaaa gccatgtgta aagttaaccc cactctgtgt tactctacat 300
tgcactaatt tgaagaatgc tactaataacc aagagtagta attggaaaga gatggacaga 360
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gaatatgcac ttttttataa acttgatgta gtaccaatag ataattgataa tacaagctat 480
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agagtcttgg ctgtggaag atacctaaag gatcaacagc tcctagggat ttggggttgc 1680

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tctggaaaac tcatttgcac cactgctgtg ccttggaatg ctagttggag taataaatct 1740
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 acaaacttaa tatacacctt aattgaagaa tcgcagaacc aacaagaaaa gaatgaacaa 1860
 gaattattag aattggataa gtgggcaagt ttgtggaatt ggtttgacat atcaaaatgg 1920
 ctgtggtata ta 1932

<210> 32

<211> 2457

<212> DNA

<213> Human immunodeficiency virus

<400> 32

gtagaaaaat tgtgggtcac agtctattat ggggtacctg tgtggaaaga agcaaccacc 60
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 gaaaatttta acatgtggaa aaataacatg gtagaacaga tgcattgagga tataatcagt 240
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 gaattatgcac ttttttataa acttgatgta gtaccaatag ataatagataa tacaagctat 480
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 gcttttctcc acatacctag aagaataaga cagggtcttg aaagggtctt gctataa 2457

<210> 33

<211> 1453

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp120.modSF162

<400> 33

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cccgtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcaa ggcctacgac 180
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acccccctgt gcgtgaccct gcaactgcacc aacctgaaga acgccaccaa caccaagagc 420
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<210> 34

<211> 1387

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp120.modSF162.delV2

<400> 34

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cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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agcacctgga acaacaccat cggccccaa aacaccaacg gcaccatcac cctgccctgc 1140

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ggcaaggaga tcagcaaacac caccgagatc ttccgccccg gcggcggcga catgcgagac 1320
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ccacca
1387

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<210> 35

<211> 1323

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp120.modSF162.delV1V2

<400> 35

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tgcacccacg gcatccgccc cgtgggtgag acccagctgc tgctgaacgg cagcctggcc 600
gaggagggcg tgggtgatccg cagcgagaac ttaccgaca acgccaagac catcatcgtg 660
cagctgaagg agagcgtgga gatcaactgc acccgcccc acaacaacac ccgcaagagc 720
atcaccatcg gccccggccg cgcttctac gccaccggcg acatcatcgg cgacatccgc 780
caggccact gcaacatcag cggcgagaag tggaaacaac cctgaagca gatcgtgacc 840
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cccgagatcg tgatgcacag cttcaactgc ggcggcgagt tcttctactg caacagcacc 960
cagctgttca acagcacctg gaacaacacc atcggcccc acaacaccaa cggcaccatc 1020
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tacgcccccc ccatccgccc ccagatcccg tgcagcagca acatcacccg cctgctgctg 1140
acccgcgacg gcggcaaggc gatcagcaac accaccgaga tcttccgccc cggcggcgcc 1200
gacatgcgcg acaactggcg cagcgagctg tacaagtaca aggtggtgaa gatcgagccc 1260
ctgggctggt ccccccacaa ggccaagcgc cgcgtggtgc agcgcgagaa gcgctaactc 1320
gag
1323

```

<210> 36

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp140.modSF162

<400> 36

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gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg ttccgcccag cgcctgggag aagctgtggg tgaccgtgta ctacggcgctg 120
cccgtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcaa ggctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag 240
gagatcgctg tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcaactgacc aacctgaaga acgccaacaa caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgaccacc 480
agcatccgca acaagatgca gaaggagtac gccctgttct acaagctgga cgtggtgccc 540
atcgacaacg acaacaccag ctacaagctg atcaactgca acaccagcgt gatcacccag 600

```

```

gcctgccccca aggtgagctt cgagcccatc cccatccact actgcgcccc cgccggcttc 660
gccatcctga agtgcaacga caagaagttc aacggcagcg gcccctgcac caacgtgagc 720
accgtgcagt gcacccacgg catccgcccc gtggtgagca cccagctgct gctgaacggc 780
agcctggccg agggggcggt ggtgatccgc agcgagaact tcaccgacaa cgccaagacc 840
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cgcaagagca tcaccatcgg ccccgggcgc gccttctacg ccaccggcga catcatcggc 960
gacatccgcc agggccactg caacatcagc ggcgagaagt ggaacaacac cctgaagcag 1020
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ggcgggcgacc ccgagatcgt gatgcacagc ttcaactgcg gcggcgagtt cttctactgc 1140
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aaggccatgt acgccccccc catccgcggc cagatccgct gcagcagcaa catcaccggc 1320
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ggcgggcgcg acatgcgcga caactggcgc agcgagctgt acaagtacaa ggtggtgaag 1440
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cgcgccgtga cctggggcgc catgttccct ggcttccctg gcgccgcccg cagcaccatg 1560
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cagcagaaca acctgctgca cgccatcagc gccacgagc acctgctgca gctgaccgtg 1680
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aacgccagct ggagcaacaa gagcctggac cagatctgga acaacatgac ctggatggag 1860
tgggagcgcg agatcgacaa ctacaccaac ctgatctaca ccctgatcga ggagagccag 1920
aaccagcagg agaagaacga gcaggagctg ctggagctgg acaagtgggc cagcctgtgg 1980
aactgggtcg acatcagcaa gtggctgtgg tacatctaac tcgag 2025

```

<210> 37

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.modSF162.delV2

<400> 37

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gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg ttctgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggagggcac caccacctg ttctgcgcca gcgacgcaa ggcttacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgacctt gcaactgcca aacctgaaga acgccaccaa caccaagagc 420
agcaactgga aggagatgga ccgcgggcag atcaagaact gcagcttcaa ggtgggcgac 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagg cctgccccaa ggtgagcttc 540
gagcccatcc ccatccacta ctgcgcccc gcccgttctg ccattcctgaa gtgcaacgac 600
aagaagtcca acggcagcgg cccctgcacc aacgtgagca ccgtgcagtg caccacggc 660
atccgccccg tggtagcac ccagctgctg ctgaacggca gcctggccga ggagggcgtg 720
gtgatccgca gcgagaactt caccgacaac cccaagacca tcatcgtgca gctgaaggag 780
agcgtggaga tcaactgcac cgcgcccaac aacaacaccc gcaagagcat caccatcggc 840
cccgcccgcg ctttctacgc caccggcgac atcatcggcg acatccgcca ggcccaactgc 900
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cagttcggca acaagaccat cgtgttcaa gacagcagcg gcggcgaccc cgagatcgtg 1020
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agcacctgga acaacaccat cggccccaac aacaccaacg gcaccatcac cctgccctgc 1140
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atccgcgccc agatccgctg cagcagcaac atcaccggcc tgctgctgac ccgcgacggc 1260
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aactggcgca gcgagctgta caagtacaag gtggtgaaga tcgagcccc gggcgtggcc 1380
cccaccaagg ccaagcgccg cgtggtgcag cgcgagaagc gcgccgtgac cctgggcgcc 1440

```

```

atgttcctgg gcttcctggg cgccgcgggc agcaccatgg gcgcccgcag cctgaccctg 1500
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gccatcgagg cccagcagca cctgctgcag ctgaccgtgt ggggcatcaa gcagctgcag 1620
gcccgcgctg tggccgtgga gcgtacctg aaggaccagc agctgctggg catctggggc 1680
tgcagcggca agctgatctg caccaccgcc gtgccctgga acgccagctg gagcaacaag 1740
agcctggacc agatctggaa caacatgacc tggatggagt gggagcgcga gatcgacaac 1800
tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggccc agcctgtgga actggttcga catcagcaag 1920
tggtctgtgt acatctaact cgag 1944

```

<210> 38

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.modSF162.delV1/V2

<400> 38

```

gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg tttcgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccggtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag 240
gagatcgctg tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct ccactgcacc aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgggccc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagc cctgccccaa ggtgagcttc 540
gagcccatcc ccattcacta ctgcgcccc gccggttcg ccattctgaa gtgcaacgac 600
aagaagtcca acggcagcgg cccctgcacc aacgtgagca ccgtgcagtg caccacggc 660
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aacatcagcg gcgagaagtg gaacaacacc ctgaagcaga tcgtgaccaa gctgcaggcc 960
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agcacctgga acaacaccat cggccccaac aacaccaacg gcaccatcac cctgccctgc 1140
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tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggccc agcctgtgga actggttcga catcagcaag 1920
tggtctgtgt acatctaact cgag 1944

```

<210> 39

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.mut.modSF162

<400> 39

```
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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
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cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcaactgcacc aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgaccacc 480
agcatccgca acaagatgca gaaggagtac gcctgtttct acaagctgga cgtggtgccc 540
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accgtgcagt gacccacgg catccgcccc gtggtgagca cccagctgct gctgaacggc 780
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cgcaagagca tcaccatcgg ccccgccgc gccttctacg ccaccggcga catcatcggc 960
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aacgccagct ggagcaacaa gagcctggac cagatctgga acaacatgac ctggatggag 1860
tgggagcgcg agatcgacaa ctacaccaac ctgatctaca ccctgatcga ggagagccag 1920
aaccagcagg agaagaacga gcaggagctg ctggagctgg acaagtgggc cagcctgtgg 1980
aactggttcg acatcagcaa gtggctgtgg tacatctaac tcgag 2025
```

<210> 40

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.mut.modSF162.delV2

<400> 40

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gcagtcttcg ttctgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgctg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
accgaggtgc acaacgtgtg ggccacccac gcctgctgct ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcaactgcacc aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtggcgccc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagg cctgccccaa ggtgagcttc 540
```



```

gagcccatcc ccatccacta ctgcgcccc gccggcttcg ccatacctgaa gtgcaacgac 600
aagaagtcca acggcagcgg cccctgcacc aacgtgagca ccgtgcagtg caccacggc 660
atccgccccg tggtagcac ccagctgctg ctgaacggca gcctggccga ggaggcgctg 720
gtgatccgca gcgagaactt caccgacaac gccaaagacca tcatcgtgca gctgaaggag 780
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gcccgcgctg tggccgtgga gcgctacctg aaggaccagc agctgctggg catctggggc 1680
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tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggccc agcctgtgga actggttcga catcagcaag 1920
tggtgtggtt acatctaact cgag
1944

```

<210> 41

<211> 1836

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modSF162.delV1/V2

<400> 41

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gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg ttctgcccag cgcgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
ccgctgtgga aggaggccac caccacctg ttctgcgcca gcgacgcaa ggcctacgac 180
accgaggtgc acaactgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag 240
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cagatgcacg aggacatcat cagcctgtg gaccagagcc tgaagccctg cgtgaagctg 360
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aaggtgagct tcgagcccat ccccatccac tactgcgccc ccgcccggctt cgccatcctg 480
aagtgaacg acaagaagtt caacggcagc ggcccctgca ccaacgtgag caccgtgcag 540
tgcacccacg gcatccgccc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc 600
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cagctgttca acagcacctg gaacaacacc atcgccccca acaacaccaa cggcaccatc 1020
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```

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gagatcgaca actacaccaa cctgatctac accctgatcg aggagagcca gaaccagcag 1740
gagaagaacg agcaggagct gctggagctg gacaagtggg ccagcctgtg gaactggttc 1800
gacatcagca agtggctgtg gtacatctaa ctcgag 1836

```

<210> 42

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut7.modSF162

<400> 42

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gcagtcttcg tttcgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcca ggccctacgac 180
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tgggagcgg agatcgacaa ctacaccaac ctgatctaca ccctgatcga ggagagccag 1920
aaccagcagg agaagaacga gcaggagctg ctggagctgg acaagtgggc cagcctgtgg 1980
aactggttcg acatcagcaa gtggctgtgg tacatctaac tcgag 2025

```

<210> 43

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.mut7.modSF162.delV2

<400> 43

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cccggtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcaa ggcctacgac 180
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gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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ggcaagctga tcaactgcaa caccagcgtg atcaccagg octgcccacaa ggtgagcttc 540
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tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggc agcctgtgga actggttcga catcagcaag 1920
tggtgtggt acatctaact cgag 1944

```

<210> 44

<211> 1836

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.mut7.modSF162.delV1/V2

<400> 44

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aagtgaacg acaagaagtt caacggcagc ggcccctgca ccaacgtgag caccgtgcag 540
tgcaccacg gcatccgccc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc 600
gaggagggcg tggatgatcc cagcgagaac ttcaccgaca acgccaagac catcatcgtg 660

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caggcccaact gcaacatcag cggcgagaag ttggaacaaca ccctgaagca gatcgtgacc 840
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accctgccct gccgcatcaa gcagatcatc aaccgctggc aggaggtggg caaggccatg 1080
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gagaagaacg agcaggagct gctggagctg gacaagtggg ccagcctgtg gaactggttc 1800
gacatcagca agtggctgtg gtacatctaa ctcgag 1836

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<210> 45

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut8.modSF162

<400> 45

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cccggtgtgga aggaggccac caccaccctg ttctgcccga gcgacgcca_ ggctacgac 180
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gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
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agcaactgga aggagatgga ccgcgcgag atcaagaact gcagcttcaa ggtgaccacc 480
agcatccgca acaagatgca gaaggagtag gccctgttct acaagctgga cgtgggtgcc 540
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ggcgcccgca gcctgacctt gaccgtgcag gcccggcagc tgctgagcgg catcgtgcag 1620
cagcagaaca acctgctgcg gcccatcagc gcccagcagc acctgctgca gctgaccgtg 1680

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tggggcatca agcagctgca ggcccgcgtg ctggccgtgg agcgtacct gaaggaccag 1740
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aacgccagct ggagcaacaa gagcctggac cagatctgga acaacatgac ctggatggag 1860
tgggagcgcg agatcgacaa ctacaccaac ctgatctaca ccctgatcga ggagagccag 1920
aaccagcagg agaagaacga gcaggagctg ctggagctgg acaagtgggc cagcctgtgg 1980
aactggttcg acatcagcaa gtggctgtgg tacatctaac tcgag 2025

```

<210> 46

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.mut8.modSF162.delV2

<400> 46

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcctacgac 180
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caggagctgc tggagctgga caagtgggcc agcctgtgga actggttcga catcagcaag 1920
tggctgtggt acatctaact cgag 1944

```

<210> 47

<211> 1836

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.mut8.modSF162.delV1/V2

<400> 47

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gagaagaacg agcaggagct gctggagctg gacaagtggg ccagcctgtg gaactggttc 1800
gacatcagca agtggctgtg gtacatctaa ctcgag 1836
```

<210> 48

<211> 2547

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp160.modSF162

<400> 48

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agcaactgga aggagatgga ccgcgggcag atcaagaact gcagcttcaa ggtgaccacc 480
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ctgcgcgacc tgatcctgat cgcgcggcgc atcgtggagc tgctggggcg ccgcggctgg 2340
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gccgtgagcc tggtcgagc catcgccatc gccgtggccg agggcaccga ccgcatcatc 2460
gaggtggccc agcgcacatc ccgcgccttc ctgcacatcc cccgcgcgat ccgccagggc 2520
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<210> 49

<211> 2466 -

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp160.modSF162.delV2

<400> 49

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cccggtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcca ggcctacgac 180
accgaggtgc acaacgtgtg ggccaccac cctgcgtgca ccaccgacc caaccccag 240
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cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgacctt gcaactgcac aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcgggcag atcaagaact gcagcttcaa ggtgggcgcc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagg cctgccccaa ggtgagcttc 540
gagcccatcc ccatccacta ctgcgcccc gccggcttcg ccctcctgaa gtgcaacgac 600
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atccgccccg tgggtgagcac ccagctgctg ctgaacggca gcctggccga ggagggcgtg 720
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accgtgcagg cccgccagct gctgagcggc atcgtgcagc agcagaacaa cctgctgcgc 1560
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ctcgag
2466

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<210> 50

<211> 2358

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp160.modSF162.delV1/V2

<400> 50

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcctacgac 180
accgaggtgc acaacgtgtg ggccacccac gcctgctgct ccaccgacct caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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aagtgaacg acaagaagtt caacggcagc ggccccctga ccaacgtgag caccgtgcag 540
tgcacccacg gcatccgccc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc 600
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ggcatctggg gctgcagcgg caagctgac tgcaccaccg ccgtgccctg gaacgcccag 1620
tggagcaaca agagcctgga ccagatctgg aacaacatga cctggatgga gtgggagcgc 1680

```



```

gagatcgaca actacaccaa cctgatctac accctgatcg aggagagcca gaaccagcag 1740
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cagcgcacgt gccgcgcctt cctgcacatc ccccgccgca tccgccaggg cttcgagcgc 2340
gcctgtctgt aactcgag                                     2358

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<210> 51

<211> 1494

<212> DNA

<213> Human immunodeficiency virus

<400> 51

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acacatgcct gtgtacccac agacccaac ccacaggaag taaatttaac aaatgtgaca 180
gaaaatttta acatgtggaa aaataacatg gtggaacaga tgcagtagga tataatcagt 240
ttatgggata aaagcctaaa gccatgtgta aaattaaccc cactctgtgt tacttttaat 300
tgtactgata agttgacagg tagtactaat ggcacaaata gtactagtgg cactaatagt 360
actagtggca ctaatagtac tagtactaat agtactgata gttgggaaaa gatgccagaa 420
ggagaaataa aaaactgctc tttcaatata accacaagt taagagataa agtgcagaaa 480
gaatattctc tcttctataa acttgatgta gtaccaatag ataataata tgctagctat 540
agattgataa attgtaatac ctcatgctt acacaagcct gtccaaagg atcttttgaa 600
ccaattccca tacattattg tgccccggct ggttttgcca ttctaaagt taaagataag 660
aagtccaatg gaacaggacc atgtaaaaat gtcagcacag tacaatgcac acatggaatt 720
agaccagtag tatcaactca actgctgtta aatggcagtc tagcagaaga agagatagta 780
cttagatctg aaaatttcac agacaatgct aaaaccataa tagtacagct gaatgaatct 840
gtagaaatta attgtataag acccaacaat aatacaagaa aaagtataca tataggacca 900
gggagagcat tttatgcaac aggtgatata ataggagaca taagacaagc acattgtaac 960
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cctcccatca gaggacaaat taaatgttca tcaaatatta cagggtgtgt attaactaga 1320
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aacatgaagg acaattggag aagtgaatta tataaatata aagtagtaag aattgaacca 1440
ttaggagtag caccaccca ggcaaagaga agagtgggtg aaagagagaa aaga 1494

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<210> 52

<211> 2007

<212> DNA

<213> Human immunodeficiency virus

<400> 52

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acacatgcct gtgtacccac agacccaac ccacaggaag taaatttaac aaatgtgaca 180
gaaaatttta acatgtggaa aaataacatg gtggaacaga tgcagtagga tataatcagt 240
ttatgggata aaagcctaaa gccatgtgta aaattaaccc cactctgtgt tacttttaat 300
tgtactgata agttgacagg tagtactaat ggcacaaata gtactagtgg cactaatagt 360
actagtggca ctaatagtac tagtactaat agtactgata gttgggaaaa gatgccagaa 420
ggagaaataa aaaactgctc tttcaatata accacaagt taagagataa agtgcagaaa 480

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ccaattccca tacattattg tgccccggtt ggttttgca ttctaaagt taaagataag 660
aagttcaatg gaacaggacc atgtaaaaat gtcagcacag tacaatgcac acatggaatt 720
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gggagagcat tttatgcaac aggtgatata ataggagaca taagacaagc acattgtaac 960
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tggagtaata aatctctgac tgagatttgg gataaatatga cctggatgga gtgggaaaga 1860
gaaattggca attatatac cttaatatag aatttaattg aaatagcaca aaaccagcaa 1920
gaaaagaatg aacaagaatt attggaatta gacaagtggg caagtttgtg gaattggttt 1980
gatataacaa actggctgtg gtatata 2007

```

<210> 53

<211> 2532

<212> DNA

<213> Human immunodeficiency virus

<400> 53

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acacatgcct gtgtaccacac agacccaac ccacaggaag taaatttaac aaatgtgaca 180
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actagtggca ctaatagtac tagtactaat agtactgata gttgggaaaa gatgccagaa 420
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gaatattctc tcttctataa acttgatgta gtaccaatag ataatgataa tgctagctat 540
agattgataa attgtaatac ctcagtcatt acacaagcct gtccaaaggt atcttttgaa 600
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gtagaaatta attgtataag acccaacaat aatacaagaa aaagtataca tataggacca 900
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gctttactat aa 2532

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<210> 54

<211> 1599

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp120.modUS4

<400> 54

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gagaccttcc gccccggcgg cggcaacatg aaggacaact ggcgcagcga gctgtacaag 1500
tacaaggtgg tgcgcatcga gcccctgggc gtggccccc cccaggccaa gcgcgcgtg 1560
gtgcagcgcg agaagcgcta agatatcgga tcctctaga 1599

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<210> 55

<211> 1350

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp120.modUS4.del 128-194

<400> 55

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<210> 56

<211> 2112

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp140.modUS4

<400> 56

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<210> 57

<211> 2112

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modUS4

<400> 57

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cccggttgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccacc gcctgcgtgc ccaccgacc caacccccag 240
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cagatgcctg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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atcgagatcg cccagaacca gcaggagaag aacgagcagg agctgctgga gctggacaag 2040
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 ggaatccteta ga 2112

<210> 58

<211> 2181

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp140TM.modUS4

<400> 58

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<210> 59

<211> 1818

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
 gp140.modUS4.delV1/V2

<400> 59

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<210> 60

<211> 2031

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.modUS4.delV2

<400> 60

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca ggcacgcca ggcttacaag 180
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<210> 61

<211> 1818

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.mut.modUS4.delV1/V2

<400> 61

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tgcaccacca ccgtgccttg gaacagcagc tggagcaaca agagcctgac cgagatctgg 1620
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aacctgatcg agatcgcccc gaaccagcag gagaagaacg agcaggagct gctggagctg 1740
gacaagtggg ccagcctgtg gaactggttc gacatcacca actggctgtg gtacatctaa 1800

```


gatatcggat cctctaga

1818

<210> 62

<211> 1818

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.modUS4.del 128-194

<400> 62

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gcagtcttcg tttcgcccag cgccaccacc gtgctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
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cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgggcgcc 360
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aacggcagcc tggcgagga ggagatcgtg ctgcgtccg agaacttcac cgacaacgcc 600
aagaccatca tcgtgcagct gaacgagtcg gtggagatca actgcatccg cccaacaac 660
aacacgcgta agagcatcca catcgcccc ggccgcgcct tctacgccac cggcgacatc 720
atcggcgaca tccgccaggc ccactgcaac atcagcaagg ccaactggac caacaccctc 780
gagcagatcg tggagaagct gcgcgagcag ttccgcaaca acaagaccat catcttcaac 840
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gatatcggat cctctaga 1818
```

<210> 63

<211> 1863

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modUS4.del 128-194

<400> 63

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gcagtcttcg tttcgcccag cgccaccacc gtgctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccacc gcctgcgtgc ccaccgacc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
```

```

cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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aaggtgagct tcgagcccat ccccatccac tactgcgccc ccgcccggctt cgccatcctg 480
aagtgcagg acaagaagtt caacggcacc ggcccctgca agaacgtgag caccgtgcag 540
tgacccacg gcatccgccc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc 600
gaggaggaga tcgtgctgcg ctccgagAAC ttcaccgaca acgccaagac catcatcgtg 660
cagctgaacg agtccgtgga gatcaactgc atccgcccc acaacaacac gcgtaagagc 720
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caggccact gcaacatcag caaggccaac tggaccaaca ccctcgagca gatcgtggag 840
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atggagtggg agcgcgagat cggcaactac accggcctga tctacaacct gatcgagatc 1740
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ctgtggaaact ggttcgacat caccaactgg ctgtggtaca tctaagatat cggatcctct 1860
aga

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1863

<210> 64

<211> 2634

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp160.modUS4

<400> 64

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccacc gcctgcgtgc ccaccgacc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgacctt gaactgcacc gacaagctga ccggcagcac caacggcacc 420
aacagcacca gcggcaccaa cagcaccagc ggcaccaaca gcaccagcac caacagcacc 480
gacagctggg agaagatgcc cgaggggcag atcaagaact gcagcttcaa catcaccacc 540
agcgtgcgcg acaagtgca gaaggagtac agcctgttct acaagctgga cgtggtgccc 600
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gcctgcccc aagtgagctt cgagcccatc cccatccact actgcgcccc cgccggcttc 720
gccatcctga agtgcaagga caagaagttc aacggcaccg gccctgcaa gaacgtgagc 780
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atcatcgtgc agctgaacga gtccgtggag atcaactgca tccgccccaa caacaacacg 960
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agcggcggcg accccgagat cgtgttccac agcttcaact gcggcgcgga gttcttctac 1200
tgcaacacca gccagctggt caacagcacc tggaaatca ccgaggaggt gaacaagacc 1260
aaggagaacg acaccatcat cctgcctgce cgcacccgca agatcatcaa catgtggcag 1320

```

```

gagggtgggca agggcatgta cggccccccc atccgcggcc agatcaagtg cagcagcaat 1380
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gagaccttcc gccccggcgg cggcaacatg aaggacaact ggcgcagcga gctgtacaag 1500
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accgaccgca tcacgcagat cgtgcagcgc atcttcgcgc ccgtgatcca catccccgc 2580
cgcacccgcc agggcctgga gcgcgcctg ctgtaagata tcggatcctc taga 2634

```

<210> 65

<211> 2538

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp160.modUS4.delV1

<400> 65

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cccggtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
gccgaggccc acaacgtgtg ggcacccac gcctgcgtgc ccaccgacc caaccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgacctt gaactgcacc gacaagctgg gcgccggcgg cgagatcaag 420
aactgcagct tcaacatcac caccagcgtg cgcgacaagg tgcagaagga gtacagcctg 480
ttctacaagc tggacgtggt gcccacgcac aacgacaacg ccagctaccg cctgatcaac 540
tgcaaacacca gcgtgatcac ccaggcctgc cccaaggtga gcttcgagcc catccccatc 600
cactactgcg cccccgcggg ctctgccatc ctgaagtgcaggacaagaa gttcaacggc 660
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agcaccacag tgctgtgtaa cggcagcctg gccgaggagg agatcgtgtg gcgctccgag 780
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aactgcggcg gcgagttctt ctactgcaac accagccagc tgttcaacag cacctggaac 1140
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cccaccagg ccaagcgccg cgtggtgcag cgcgagaagc gcgcctggg cctgggccc 1500
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```

```

accgtgcagg cccgccagct gctgagcggc atcgtgcagc agcagaacaa cctgctgcgc 1620
gccatcgagg cccagcagca cctgctgcag ctgaccgtgt ggggcatcaa gcagctgcag 1680
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cgcgccgtga tccacatccc ccgccgcac cgcaggggc tggagcgcgc cctgctgtaa 2520
gatatcgcat cctctaga
2538

```

<210> 66

<211> 2553

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp160.modUS4.delV2

<400> 66

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cccggtgtgga aggaggccac caccacctg ttctgcgcca gcagcgccaa ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccac gcctgcgtgc ccaccgacc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catgggtggg 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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aacagcacca gcggcaccac cagcaccagc ggcaccaaca gcaccagcac caacagcacc 480
gacagctggg agaagatgcc cgagggcgag atcaagaact gcagcttcaa catcggcgcc 540
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atccgccccg tggtagcac ccagctgtg ctgaacggca gcctggccga ggaggagatc 780
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agctggagca acaagagcct gaccgagatc tgggacaaca tgacctggat ggagtgggag 1860

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```

cgcgagatcg gcaactacac cggcctgata tacaacctga tcgagatcgc ccagaaccag 1920
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cgcgccctgc tgtaagatat cggatcctct aga

```

<210> 67

<211> 2340

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp160.modUS4.delV1/V2

<400> 67

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cccgtgtgga aggagggcac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccacc gcctgcgtgc ccaccgaccc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgggcgcy 360
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aagaccatca tcgtgcagct gaacgagtcg gtggagatca actgcatccg cccaacaac 660
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gagcagatcg tggagaagct gcgcgagcag ttccggcaaca acaagaccat catcttcaac 840
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cagcgcgccc ccgaccgccc cgagggcac gcaggagag gcggcgagcy cgaccgcgac 1980
cgcgcaaac gcctggtgca cggcctgctg gccctgatct gggacgacct gcgcagcctg 2040
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agccaggagc tgaagagcag cgccgtgagc ctgttcaacg ccaccgccat cgccgtggcc 2220
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 ccccgccgca tccgccaggg cctggagcgc gccctgctgt aagatatcgg atcctctaga 2340

<210> 68

<211> 2385

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp160.modUS4del 128-194

<400> 68

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 cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
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<210> 69

<211> 144

<212> DNA

<213> Human immunodeficiency virus

<400> 69

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 aaggccatgt acgccccccc catccgcggc cagatcaagt gcagcagcaa catcaccggc 120
 ctgctgctga cccgcgacgg cggc 144

<210> 70

<211> 144

<212> DNA

<213> Human immunodeficiency virus

<400> 70

ggaactatca cactcccatg cagaataaaa caaattataa acaggtggca ggaagtagga 60
 aaagcaatgt atgccccctc catcagagga caaattagat gctcatcaaa tattacagga 120
 ctgtatttaa caagagatgg tggc 144

<210> 71

<211> 144

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic Env
 US4 common region

<400> 71

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 aaggccatgt acgccccccc catccgcggc cagatcaagt gcagcagcaa catcaccggc 120
 ctgctgctga cccgcgacgg cggc 144

<210> 72

<211> 144

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic Env
 SF162 common region

<400> 72

ggcaccatca ccctgccctg ccgcatcaag cagatcatca accgctggca ggaggtgggc 60
 aaggccatgt acgccccccc catccgcggc cagatccgct gcagcagcaa catcaccggc 120
 ctgctgctga cccgcgacgg cggc 144

<210> 73

<211> 4766

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
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<400> 73

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 cccgtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcaa ggcttacaag 180
 gccgaggccc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
 gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
 cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360

| | | | | | | |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| acccccctgt | gcgtgacctt | gaactgcacc | gacaagctga | ccggcagcac | caacgggcacc | 420 |
| aacagcacca | gcggcaccaa | cagcaccagc | ggcaccaaca | gcaccagcac | caacagcaccc | 480 |
| gacagctggg | agaagatgcc | cgaggcgag | atcaagaact | gcagcttcaa | catcaccacc | 540 |
| agcgtgcgcg | acaaggtgca | gaaggagtac | agcctgttct | acaagctgga | cgtggtgccc | 600 |
| atcgacaacg | acaacgccag | ctaccgcctg | atcaactgca | acaccagcgt | gatcacccag | 660 |
| gcttgcccca | aggtgagctt | cgagcccatc | cccatccact | actgcgcccc | cgccggcttc | 720 |
| gccatcctga | agtgaagga | caagaagttc | aacggcaccc | gccctgcaa | gaacgtgagc | 780 |
| accgtgcagt | gcacccacgg | catccgcccc | gtggtgagca | cccagctgct | gctgaacggc | 840 |
| agcctggccg | aggaggagat | cgtgctgcgc | tccgagaact | tcaccgacaa | cgccaagacc | 900 |
| atcatcgtgc | agctgaacga | gtccgtggag | atcaactgca | tccgccccaa | caacaacacg | 960 |
| cgtaagagca | tccacatcgg | ccccggccgc | gccttctacg | ccaccggcga | catcatcggc | 1020 |
| gacatccgcc | aggcccaactg | caacatcagc | aaggccaact | ggaccaacac | cctcgagcag | 1080 |
| atcgtggaga | agctgcgcga | gcagttcggc | aacaacaaga | ccatcatctt | caacagcagc | 1140 |
| agcggcgccg | accccgagat | cgtgttccac | agcttcaact | gcggcgccga | gttcttctac | 1200 |
| tgaacacca | gccagctggt | caacagcacc | tggaaacatca | ccgaggaggt | gaacaagacc | 1260 |
| aaggagaacg | acaccatcat | cctgccctgc | cgcatccgcc | agatcatcaa | catgtggcag | 1320 |
| gaggtgggca | aggccatgta | cgcccccccc | atccgcgccc | agatcaagtg | cagcagcaat | 1380 |
| attaccggcc | tgtctgtgac | ccgcgacggc | ggcaccaaca | acaaccgcac | caacgcacac | 1440 |
| gagaccttcc | gccccggcgg | ggcaacatg | aaggacaact | ggcgagcga | gctgtacaag | 1500 |
| tacaaggtgg | tgcgcatcga | gccccggg | gtggccccc | cccaggccaa | gcgcgcgctg | 1560 |
| gtgcagcgcg | agaagcgcg | cgtgggcctg | ggcgccctgt | tcatcggtt | cctgggcgccc | 1620 |
| gcccgggagca | ccatgggcgc | cgctcccgctg | accctgacgc | tgcaggcccc | ccagctgctg | 1680 |
| agcggcatcg | tgcagcagca | gaacaacctg | ctgcgcgcca | tcgaggccca | gcagcacctg | 1740 |
| ctgcagctga | cgtgtgggg | catcaagcag | ctgcaggccc | gcacctggc | cgtggagcgc | 1800 |
| tacctgaagg | accagcagct | gctgggcac | tggggctgca | gcggcaagct | gatctgcacc | 1860 |
| accacccgtg | cctggaacag | cagctggagc | aacaagagcc | tgaccgagat | ctgggacaac | 1920 |
| atgacctgga | tggagtggga | gcgcgagatc | ggcaactaca | ccggcctgat | ctacaacctg | 1980 |
| atcgagatcg | cccagaacca | gcaggagaag | aacgagcagg | agctgctgga | gctggacaag | 2040 |
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| atcatgatcg | tggggggcct | gatcggcctg | cgcatcgtgt | tcgccgtgct | gagcatcgtg | 2160 |
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| accgaccgca | tcatcgagat | cgtgcagcgc | atcttccgcg | ccgtgatcca | catccccgc | 2580 |
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| cgcccccccc | cccccccccc | ctctccctcc | ccccccccta | acgttactgg | ccgaagccgc | 2700 |
| ttggaataag | gcccgtgtgc | gtttgtctat | atgttatttt | ccaccataatt | gccgtctttt | 2760 |
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| tccctctctg | ccaaaggaat | gcaaggtctg | ttgaatgtcg | tgaaggaaag | agttcctctg | 2880 |
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| tcaagcgtat | tcaacaagg | gctgaaggat | gcccagaagg | tacccatttg | tatgggatct | 3120 |
| gatctggggc | ctcggtgcac | atgctttaca | tgtgtttagt | cgagggttaa | aaaacgtcta | 3180 |
| ggccccccga | accacgggga | cgtgggtttc | ctttgaaaaa | cacgataata | ccatgggcgc | 3240 |
| ccgcgccagc | gtgctgagcg | gcggcgagct | ggacaagtgg | gagaagatcc | gcctgcgccc | 3300 |
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| cttcgccgtg | aaccccgccc | tgtggagac | cagcgagggc | tgccgcccga | tcctgggcca | 3420 |
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| ccaggacatg | aacacgatgt | tgaacaccgt | gggcggccac | caggccgcca | tgcagatgct | 3840 |
| gaaggagacc | atcaacagg | aggccgcga | gtgggaccgc | gtgcaccccg | tgcacgcccg | 3900 |


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agaattcaga ctcgagcaag tctaga 4766

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<210> 74

<211> 4689

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp160.modSF162.gag.modSF2

<400> 74

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```

<210> 75

<211> 4472

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
 gp160.modUS4.delV1/V2.gag.modSF2

<400> 75

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gcagtcttcg ttctgcccag cgccaccacc gtgctgtggg tgaccgtgta ctacggcgtg 120

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cccggtgtgga aggaggccac caccaccctg ttctgcgcca ggcagccaa ggcttacaag 180
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gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
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<210> 76

<211> 4608

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
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<400> 76

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<210> 77

<211> 1680

<212> DNA

<213> Human immunodeficiency virus

<400> 77

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tttgctataa agaaaaaaga cagtactaaa tggagaaaac tagtagattt cagagaactt 240
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gggattagat atcagtacaa tgtgctgcca cagggtatgga aaggatcacc agcaatattc 480
caaagtagca tgacaaaaat cttagagcct tttagaaaac agaattccaga catagttatc 540

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<210> 78

<211> 1865

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: GP1

<400> 78

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aacgacccct cgtcacagta aggatcggc gccagctcaa ggaggcgctg ctcgacaccg 1560
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tcggcgggat cgggggcttc atcaaggtgc ggcagtacga ccagatcccc gtggagatct 1680
ggggccacaa ggccatcgcc accgtgctgg tgggcccac ccccgtaac atcatcgccc 1740
gcaacctgct gaccagatc ggctgcaccc tgaacttccc catcagcccc atcgagacgg 1800

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tgccccgtgaa gctgaagccg gggatggacg gcccccaaggt caagcagtgg cccctgtaag 1860
aattc 1865

<210> 79

<211> 1865

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: GP2

<400> 79

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gccagcccg agctggagcg cttcgccgtg aaccccgcc tgctggagac cagcgagggc 180
tgccgccaga tcctggggcca gctgcagccc agcctgcaga ccggcagcga ggagctgcgc 240
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accaaggagg ccctggagaa gatcgaggag gagcagaaca agtccaagaa gaaggcccag 360
caggccgccc ccgcccgcgg caccggcaac agcagccagg tgagccagaa ctaccccatc 420
gtgcagaacc tgcagggcca gatggtgcac caggccatca gccccgcac cctgaacgcc 480
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ctgagcgagg gcgcaccccc ccaggacctg aacacgatgt tgaacaccgt gggcgccac 600
caggccgcca tgcagatgct gaaggagacc atcaacgagg agggcgccga gtgggaccgc 660
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tgcccgtaaa gttgaagccg gggatggacg gcccccaaggt caagcaatgg ccattgtaag 1860
aattc 1865

<210> 80

<211> 2305

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

FS(+).proinact.RTopt.YM

<400> 80

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agatctggcc ttctacaag ggaaggccag ggaattttct tcagagcaga ccagagccaa 120
cagccccacc agaagagagc ttcaggtttg gggaggagaa aacaactccc tctcagaagc 180
aggagccgat agacaaggaa ctgtatcctt taacttcctt cagatcactc tttggcaacg 240

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accctcgtc acaataagga tcggggggca actcaaggaa gcgctgctcg atacaggagc 300
agatgataca gtattagaag aaatgaattt gccaggaaaa tggaaaccaa aaatgatagg 360
ggggatcggg ggcttcatca aggtgaggca gtacgaccag atacctgtag aaatctgtgg 420
acataaagct ataggtacag tattagtagg acctacacct gtcaacataa ttggaagaaa 480
tctgttgacc cagatcggct gcaccttgaa ctccccatc agccctattg agacggtgcc 540
cgtgaagttg aagccgggga tggacggccc caaggtcaag caatggccat tgaccgagga 600
gaagatcaag gccctggtgg agatctgcac cgagatggag aaggagggca agatcagcaa 660
gatcggcccc gagaaccctt acaacacccc cgtgttcgcc atcaagaaga aggacagcac 720
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caccaccaac cagaagaccg agctgcaggc catccacctg gccctgcagg acagcggcct 1980
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ggtgagcgcc ggcattccga aggtgctgtt cctgaacggc atcgatggcg gcacgtgat 2220
ctaccagtac atggacgacc tgtacgtggg cagcggcggc cctaggatcg attaaaagct 2280
tccccgggct agcaccgggtg aattc 2305

```

<210> 81

<211> 2299

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

FS(+) .proinact.RTopt.YMWM

<400> 81

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gcggccgcga aggacaccaa atgaaagatt gcactgagag acaggctaatt tttttaggga 60
agatctggcc ttctacaag ggaaggccag ggaattttct tcagagcaga ccagagccaa 120
cagccccacc agaagagagc ttcagggttg gggaggagaa aacaactccc tctcagaagc 180
aggagccgat agacaaggaa ctgtatcctt taacttccct cagatcactc tttggcaacg 240
accctcgtc acaataagga tcggggggca actcaaggaa gcgctgctcg atacaggagc 300
agatgataca gtattagaag aaatgaattt gccaggaaaa tggaaaccaa aaatgatagg 360
ggggatcggg ggcttcatca aggtgaggca gtacgaccag atacctgtag aaatctgtgg 420
acataaagct ataggtagg tattagtagg acctacacct gtcaacataa ttggaagaaa 480
tctgttgacc cagatcggct gcaccttgaa ctccccatc agccctattg agacggtgcc 540
cgtgaagttg aagccgggga tggacggccc caaggtcaag caatggccat tgaccgagga 600
gaagatcaag gccctggtgg agatctgcac cgagatggag aaggagggca agatcagcaa 660
gatcggcccc gagaaccctt acaacacccc cgtgttcgcc atcaagaaga aggacagcac 720
caagtggcgc aagctggtgg acttccgcga gctgaacaag cgcacccagg acttctggga 780

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ggtgcagctg ggcattcccc accccgcgcg cctgaagaag aagaagagcg tgaccgtgct 840
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cgccggcatc cgcaagggtg tgttctgaa cggcatcgat ggcgccatcg tgatctacca 2220
gtacatggac gacctgtacg tgggcagcgg cggccctagg atcgattaaa agcttccccg 2280
ggctagcacc ggtgaattc 2299

```

<210> 82

<211> 2306

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

FS (-).protmod.RTopt.YM

<400> 82

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gcggccgcga aggacaccaa atgaaagatt gcaactgagag acaggctaata ttcttccgcg 60
aggacctggc cttcctgcag ggcaaggccc gcgagttcag cagcgagcag acccgcgcca 120
acagccccac ccgccgcgag ctgcaggtgt ggggcggcga gaacaacagc ctgagcgagg 180
ccggcgccga ccgccagggc accgtgagct tcaacttccc ccagatcacc ctgtggcagc 240
gccccctggt gaccatcagg atcggcgccc agctcaagga ggcgctgctc gacaccggcg 300
ccgacgacac cgtgctggag gagatgaacc tgcccgccaa gtggaagccc aagatgatcg 360
gcgggatcgg gggcttcatc aagggtgcggc agtacgacca gatccccgtg gagatctgcg 420
gccacaaggc catcggcacc gtgctggtgg gccccacccc cgtgaacatc atcggccgca 480
acctgctgac ccagatcggc tgcaccctga acttccccat cagccccatc gagacggtgc 540
ccgtgaagct gaagccgggg atggacggcc ccaaggtcaa gcagtggccc ctgaccgagg 600
agaagatcaa ggccctgggt gagatctgca ccgagatgga gaaggagggt aagatcagca 660
agatcggccc cgagaacccc tacaacaccc ccgtgttcgc catcaagaag aaggacagca 720
ccaagtggcg caagctgggt gacttccgcg agctgaacaa gcgcacccag gacttctggg 780
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tgccccaggg ctggaagggc agccccgcca tcttccagag cagcatgacc aagatcctgg 1020
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aggatgaccc cctgaccgag gaggccgagc tggagctggc cgagaaccgc gagatcctga 1440
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ccggcaagta cggccgcatg cggggcgccc acaccaacga cgtgaagcag ctgaccgagg 1620
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tctaccagta catggacgac ctgtacgtgg gcagcgcgcg ccctaggatc gattaaaagc 2280
tccccggggc tagcaccggt gaattc 2306
```

<210> 83

<211> 2300

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

FS(-).protmod.RTopt.YMWM

<400> 83

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gcggccgcga aggacaccaa atgaaagatt gcactgagag acaggctaatt ttcttccgcg 60
aggacctggc cttcctgcag ggcaaggccc gcgagttcag cagcgagcag acccgcgcca 120
acagccccac ccgcccgcgag ctgcagggtgt ggggcggcga gaacaacagc ctgagcgagg 180
ccggcgccga ccgccagggc accgtgagct tcaacttccc ccagatcacc ctgtggcagc 240
gccccctggt gaccatcagg atcgccggcc agctcaagga ggcgctgctc gacaccggcg 300
ccgacgacac cgtgctggag gagatgaacc tgcccggcaa gtggaagccc aagatgatcg 360
gcgggacceg gggcttcacg aaggtgcggc agtacgacca gatccccgtg gagatctgcg 420
gccacaaggc catcggcacc gtgctggtgg gccccacccc cgtgaacatc atcgcccgca 480
acctgctgac ccagatcgcc tgcacctga acttccccat cagccccatc gagacggtgc 540
ccgtgaagct gaagccgggg atggacggcc ccaaggtcaa gcagtggccc ctgaccgagg 600
agaagatcaa ggccctgggt gagatctgca ccgagatgga gaaggaggcg aagatcagca 660
agatcgcccc cgagaacccc tacaacaccc ccgtgttcgc catcaagaag aaggacagca 720
ccaagtggcg caagctggtg gacttccgcg agctgaacaa gcgcacccag gacttctggg 780
agggtgcagc gggcatcccc caccgcggcg gctgaagaa gaagaagagc gtgaccgtgc 840
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ccatcgtggg cgccgagacc ttctacgtgg acggcgccgc caaccgcgag accaagctgg 1860
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```

gcaaggccgg ctacgtgacc gaccggggcc ggcagaaggt ggtgagcatc gccgacacca 1920
ccaaccagaa gaccgagctg caggccatcc acctggccct gcaggacagc ggcctggagg 1980
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gcgcgggcat ccgcaagggt ctgttctga acggcatcga tggcggcata gtgatctacc 2220
agtacatgga cgacctgtac gtgggcagcg gcggccctag gatcgattaa aagcttccc 2280
gggctagcac cggtgaattc                                     2300

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<210> 84

<211> 2312

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

FS (-).protmod.RTopt (+)

<400> 84

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acagccccac ccgcccggag ctgcagggtgt gggcgggcga gaacaacagc ctgagcgagg 180
ccggcgccga ccgcccaggc accgtgagct tcaacttccc ccagatcacc ctgtggcagc 240
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ccgacgacac cgtgctggag gagatgaacc tgcccggcaa gtggaagccc aagatgatcg 360
gcgggatcgg gggcttcac aaggtgcggc agtacgacca gatccccgtg gagatctg 420
gccacaaggc catcggcacc gtgctggtgg gccccacccc cgtgaacatc atcgccgca 480
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ccgtgaagct gaagccgggg atggacggcc ccaagggtcaa gcagtggccc ctgaccgagg 600
agaagatcaa ggccctggtg gagatctgca ccgagatgga gaaggagggc aagatcagca 660
agatcggccc cgagaacccc tacaacaccc ccgtgttcgc catcaagaag aaggacagca 720
ccaagtggcg caagctggtg gacttccgcg agctgaacaa gcgcacccag gacttctggg 780
aggtgagct gggcatcccc caccgcggcg gcctgaagaa gaagaagagc gtgaccgtg 840
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ccttcacat cccagcatc aacaacgaga cccccggcat ccgctaccag tacaacgtg 960
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tcgtgatcta ccagtacatg gacgacctgt acgtgggcag cggcggccct aggatcgatt 2280
aaaagcttcc cggggctagc accggtgaat tc                                     2312

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<210> 85
 <211> 306
 <212> DNA
 <213> Human immunodeficiency virus

<400> 85
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 aaaggccttag gcatctccta tggcaggaag aagcggagac agcgacgaag agctcctcca 180
 gacagtggagg ttcatcaagt ttctctacca aagcaacccg cttcccagcc ccaagggggac 240
 ccgacaggcc cgaaggaatc gaagaagaag gtggagagag agacagagac agatccagtc 300
 cattag 306

<210> 86
 <211> 101
 <212> PRT
 <213> Human immunodeficiency virus

<400> 86
 Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
 1 5 10 15
 Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe
 20 25 30
 His Cys Gln Val Cys Phe Ile Thr Lys Gly Leu Gly Ile Ser Tyr Gly
 35 40 45
 Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro Pro Asp Ser Glu Val
 50 55 60
 His Gln Val Ser Leu Pro Lys Gln Pro Ala Ser Gln Pro Gln Gly Asp
 65 70 75 80
 Pro Thr Gly Pro Lys Glu Ser Lys Lys Lys Val Glu Arg Glu Thr Glu
 85 90 95
 Thr Asp Pro Val His
 100

<210> 87
 <211> 306
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: tat.SF162.opt

<400> 87
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 aagggcctgg gcatcagcta cggccgcaag aagcggcggc agcggcgccg cgcccccccc 180
 gacagcgagg tgcaccaggt gagcctgccc aagcagcccg ccagccagcc ccagggcgac 240
 cccaccggcc ccaaggagag caagaagaag gtggagcgcg agaccgagac cgaccccggtg 300
 cactag 306

<210> 88
 <211> 306

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

tat.cys22.SF162.opt

<400> 88

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aagggcctgg gcatcagcta cggccgcaag aagcgccgcc agcgccgccg cgccccccc 180
gacagcgagg tgcaccaggt gagcctgccc aagcagcccc ccagccagcc ccagggcgac 240
ccacccggcc ccaaggagag caagaagaag gtggagcgcg agaccgagac cgaccccggtg 300
cactag                                           306

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<210> 89

<211> 168

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

tatamino.SF162.opt

<400> 89

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aagggcctgg gcatcagcta cggccgcaag aagcgccgcc agcgccgcg 168

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<210> 90

<211> 102

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: tat cys22
SF162 protein

<400> 90

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Gln Pro Lys Thr Ala Gly Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe
  20          25          30

His Cys Gln Val Cys Phe Ile Thr Lys Gly Leu Gly Ile Ser Tyr Gly
  35          40          45

Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro Pro Asp Ser Glu Val
  50          55          60

His Gln Val Ser Leu Pro Lys Gln Pro Ala Ser Gln Pro Gln Gly Asp
  65          70          75          80

Pro Thr Gly Pro Lys Glu Ser Lys Lys Lys Val Glu Arg Glu Thr Glu
  85          90          95

Thr Asp Pro Val His Glx
 100

```

